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VITAMIN A DEFICIENCY AND THE NERVOUS SYSTEM

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The relation of vitamin A deficiency to lesions of the nervous system has long been an issue in a state of confusion. The solution has been found in the discovery that the deficiency when instituted at a sufficiently early age causes a disproportion between the central nervous system and its osseous envelopment. The effect is virtually that of an overgrowth of the central nervous system with consequent mechanical injuries to the brain, spinal cord and nerve roots as a result of pressure.

The substance of this paper comes chiefly from experiments on white rats. Confirmatory experiments have been made by us on guinea pigs and dogs. A review of the literature indicates that vitamin A deficiency in the young of several mammals and in fowls results in damage to the central nervous system and suggests that in all vertebrates the common consequence is that of disproportionate growth. The prediction is warranted that acute uncomplicated vitamin A deficiency in the human infant would produce similar results.

REVIEW OF PERTINENT LITERATURE

Hart, Miller and McCollum in 1916 [1] probably were the first to describe and demonstrate nerve lesions due to vitamin A deficiency, in experiments on young swine kept on wheat meal rations. Malnutrition, ataxia and paralysis were obtained and were shown to be prevented by the addition of alfalfa meal. At that time the lesions were ascribed to toxicity of the high wheat diets. Vitamin A in the alfalfa was regarded as the protective factor.

Mellanby [2] reported severe nervous symptoms and nerve lesions in puppies maintained on cereal rations as being due to toxic substances, for which he suggested the name "toxamins." He ascribed a protective function to the fat-

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1. Hart, E. B.; Miller, W. S., and McCollum, E. V.: J. Biol. Chem. **25**:239, 1916.

2. Mellanby, E.: J. Physiol. **61**:xxiv, 1926.

soluble vitamins. Later [3] he added proof that the lack of vitamin A was a causative factor in the production of the nerve lesions and that the same effects could be produced in young rabbits. He retained the belief that toxic products were also involved.

The distribution of lesions described by Mellanby in the rabbit included all the afferent nerves of the head: the optic nerves, sensory fibers of the fifth, and the auditory and vestibular fibers of the eighth nerve. In the body, also, the afferent nerves were affected, demonstrable in the dorsal roots. The anterior root fibers remained normal except when nutritional deficiency had been very prolonged. No lesions were found in the vagus nerves. In the spinal cord the ascending fibers were most involved: the dorsal and ventral spinocerebellar, the dorsal and ventral spinoreticulothalamic, and the tracts of the dorsal columns. Of descending tracts, he named the rubrospinal, the dorsal longitudinal bundle and the vestibulospinal. The pyramidal tracts generally escaped lesions.

Finally, from the study of deafness produced in puppies fed "on diets of natural foodstuffs but deficient in vitamin A and rich in cereals," Mellanby [4] reported compression of the eighth nerve by bony overgrowth of periosteal origin. He suggested that degenerative changes of other cranial nerves—the optic and the trigeminal—were due to bony overgrowth around foramens. No explanation was offered for the occurrence of nerve lesions elsewhere.

Mellanby's [4a] last publication, on the subject of nerve lesions and vitamin A deficiency in young dogs, reached us after this paper was in press. He describes overgrowth of bone in several locations—of the cranial bones, particularly those forming the posterior fossa, the vertebrae and the femurs. He regards this overgrowth as related (presumably through pressure effects) to degenerative changes in the brain and in the cranial and peripheral nerves. He states that "a function of vitamin A is to influence the structure of growing bone, probably by limiting the number and degree of activity of osteoblasts and osteoclasts. In its absence from the growing dog, osteoblastic and osteoclastic activity is increased, thus resulting in proliferation of cancellous bone at the expense of compact bone and causing many bones to lose their normally fine molding and outline and to become thickened and enlarged." He extends this idea to the epithelial changes in vitamin A deficiency by stating that the "fundamental change is overgrowth of epithelial cells of all kinds, keratinization and metaplasia being secondary to this overgrowth."

Hughes, Aubel and Lienhardt [5] reported paralysis and nerve lesions in weanling swine fed on white corn and tankage diets. Vitamin A, supplied by cod liver oil and other sources, prevented the disorder. They also reported severe nerve degeneration in chicks given vitamin A–deficient diets when a day old, and mentioned stiffness and incoordination in 3 mature cows fed a vitamin A–deficient diet. They also stated that impairment of the nervous system developed in some rats on similarly deficient diets.

Aberle, Zimmerman and Brill [6] reported paralysis and central and peripheral nerve lesions in rats nurtured on a diet low in vitamin A. The rats were given

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3. Mellanby, E.: (*a*) Brain **54**:247, 1931; (*b*) Edinburgh M. J. **40**:197, 1933; (*c*) J. Path. & Bact. **38**:391, 1934; (*d*) Brain **58**:141, 1935.

4. Mellanby, E.: J. Physiol. **94**:380, 1938.

4a. Mellanby, E.: J. Physiol. **99**:467, 1941.

5. Hughes, J. S.; Aubel, C. E., and Lienhardt, H. F.: The Importance of Vitamin A and Vitamin C in the Ration of Swine, Technical Bulletin 23, Kansas State College of Agriculture, Agricultural Experimental Station, 1928. Hughes, J. S.; Lienhardt, H. F., and Aubel, C. E.: J. Nutrition **2**:183, 1929.

6. Aberle, S. B. D.; Zimmerman, H. M., and Brill, L.: Anat. Rec. **48** (supp.): 7, 1931.

the diet at about 22 days of age. The first signs of paralysis appeared between the fifty-sixth and seventieth day of age. These rats were the basis of a careful pathologic study by Zimmerman.[7] Myelin sheath degeneration of the brachial plexuses, sciatic nerves and less frequently of the vagus nerves was found, but not of the optic nerves. In the spinal cord, myelin sheath degeneration of the sensory tracts on the periphery of the cord and in the posterior columns and occasionally in the cross and direct pyramidal tracts was found. The posterior nerve roots and less frequently the anterior nerve roots showed myelin sheath degeneration and "evidence was adduced to indicate that the changes in the sensory tracts of the spinal cord followed those in the posterior nerve roots."

Subsequent studies by Aberle[8] and Zimmerman and Cowgill[9] showed that the paralysis, while preventable by carotene or cod liver oil, could not be cured, although treatment restored a normal rate of growth.

Elvehjem and Neu[10] suggested that the staggering gait and general incoordination observed in vitamin A–deficient chicks might be due to nerve lesions. Six weeks old as well as day old chicks responded equally well.

Setterfield and Sutton[11] described ataxia and paralysis in vitamin A–deficient rats and used polarized light for the study of myelin sheath degeneration. Changes in the nerves could be demonstrated three to six days before symptoms appeared and could be arrested by giving adequate doses of vitamin A.

Irving and Richards[12] reported the constant production of nervous signs and nerve lesions in vitamin A–deficient rats. The rats were given the deficient diet at weaning. Lesions developed earlier in the rats which had access only to A-deficient food before they were weaned. The authors disproved that there was a possible toxic effect of cereals. Many of the rats showed marked nerve lesions while still gaining in weight. Female rats showed nerve lesions later than did males.

The first lesions (demyelination) occurred in the medulla in the funiculus praedorsalis at the level of the decussation of the pyramids. From this site the lesions extended higher in the medulla and down into the cervical cord. The funiculus praedorsalis, according to Irving and Richards, corresponds to the tectospinal tract in man and is a motor tract. Later, lesions of the spinocerebellar tract and the posterior columns (especially Burdach's column) developed.

Moore, Huffman and Duncan[13] reported blindness and paralysis in vitamin A-deficient calves, including calves born of vitamin A–deficient cows and calves fed on vitamin A–deficient rations. The blindness was due to bone overgrowth in the optic foramen causing stenosis and degeneration of the optic nerve by pressure.

Moore[14] extended and confirmed these observations and showed that the disorders could be prevented by adding crystalline carotene in cottonseed oil to the diet. Then Moore and Sykes[15] reported elevation of cerebrospinal fluid

7. Zimmerman, H. M.: J. Exper. Med. **57**:215, 1933.
8. Aberle, S. B. D.: J. Nutrition **7**:445, 1934.
9. Zimmerman, H. M., and Cowgill, G. R.: J. Nutrition **11**:411, 1936.
10. Elvehjem, C. A., and Neu, V. F.: J. Physiol. **97**:71, 1932.
11. Setterfield, H. E., and Sutton, T. S.: J. Nutrition **9**:645, 1935.
12. Irving, J. T., and Richards, M. B.: J. Physiol. **89**:2 P, 1937; **94**:807, 1938.
13. Moore, L. A.; Huffman, C. F., and Duncan, C. W.: J. Nutrition **9**:533, 1935.
14. Moore, L. A.: J. Nutrition **17**:443, 1939.
15. Moore, L. A., and Sykes, J. F.: Am. J. Physiol. **130**:684, 1940.

pressure in vitamin A–deficient calves and the development of papilledema. They suggested that the bone changes reported by Mellanby, affecting the eighth nerve, were related "in some manner to those reported in calves where a constriction of the optic nerve develops." They did not relate the bone changes to the elevation of cerebrospinal fluid pressure.

Phillips and Bohstedt,[16] by using diets similar to those employed by Moore and co-workers, produced in rabbits ataxia and paralysis and other signs of vitamin A deficiency but not stenosis of the optic foramen.

The best account of nerve lesions in vitamin A deficiency in fowls is that of Seifried.[17] Chicks 15 to 18 days old were largely used and gave very constant results; nervous manifestations appeared in about four months. Fowls 4 and 6 months old responded less regularly. Of 14 fowls given the deficient diet at 6 months of age, 5 exhibited evidence of nerve disorders. Ataxia, incoordination and convulsions were the manifestations. The lesions reported by Seifried were degeneration of ganglion cells in the anterior horns of the spinal cord and of the motor cortex, the nucleus dentatus and nuclei of the medulla, and of the Purkinje cells of the cerebellum. Demyelination was found in the spinal cord (tracts not stated) and in the brachial, sciatic and optic nerves.

THE PRESENT INVESTIGATION

The importance of early age and more specifically of an early period of growth as the essential factor in the production of nerve lesions in vitamin A deficiency came to be realized late in our studies, although clearly indicated in the data of the papers just reviewed. Likewise, an analysis of papers reporting failure to produce nerve lesions in rats,[18] dogs [19] and monkeys [20] reveals that the animals used had passed their early period of rapid growth.

Our studies, which have extended over a period of four years, were undertaken to answer the following questions:

1. What is the relation of vitamin A deficiency to nerve lesions in view of the conflicting observations recorded?

2. Are the epithelial changes of vitamin A deficiency secondary to nerve lesions, as suggested by Mellanby? [3c]

In the light of the solution of our problems it does not seem warranted to present in detail our initial fairly elaborate experiments in which rats and guinea pigs of considerable size were used. Normal and undernourished controls were observed in each experiment. Paralysis was not obtained. The epithelial changes of vitamin A deficiency were constant, and studies of nerves failed to show myelin sheath degenera-

16. Phillips, P. H., and Bohstedt, G.: J. Nutrition **15**:309, 1938.

17. Seifried, O.: Arch. f. wissensch. u. prakt. Tierh. **65**:140, 1932.

18. Wolbach, S. B., and Howe, P. R.: J. Exper. Med. **42**:753, 1925. Duncan, D.: Arch. Neurol. & Psychiat. **25**:327, 1931.

19. Suzman, M. M.; Muller, G. L., and Ungley, C. C.: Am. J. Physiol. **101**: 529, 1932. Eveleth, D. F., and Biester, H. E.: Am. J. Path. **13**:257, 1937.

20. Grinker, R. R., and Kandel, E.: Arch. Neurol. & Psychiat. **30**:1287, 1933.

tion in the spinal cord or in the brachial, sciatic and trigeminal nerves. (The modified Marchi technic of Swank and Davenport [21] was employed among other methods and was applied by Dr. Swank, who was associated with us during the first period of this work.)

The answer to the second question was emphatic and decisive. The epithelial changes characteristic of vitamin A deficiency were invariably produced in a large number of animals without demonstrable nerve changes.[22]

Observations which were the outcome of testing several strains of white rats from different laboratories with a few positive results led to the use of weanling rats and finally to the discovery that paralysis and nerve lesions invariably occur if the deficiency is established before the eighth week of life.

DIET AND PROCEDURES

Our stock colony is reared on Purina dog chow. The vitamin A–free diet is as follows:

	Per Cent
Casein	18
Osborn and Mendel salt mixture	3
Brewers' yeast	10
Peanut oil	8
Corn starch	60.9
Viosterol	0.1

In order to secure the early establishment of the deficiency, the mother and her young were given the diet fourteen days after birth. Large litters (8 to 12) were selected as often as possible because they show the development of neurologic signs earlier, presumably because each rat receives less vitamin A from the mother's milk. The young rats were weaned at 21 days of age and maintained on the vitamin A–deficient diet.

COURSE AND SYMPTOMS

About 500 rats were used in this study. The rats on the deficient diets continued to grow at a normal rate into the sixth week of age. Stationary weight or a loss of weight was usual in the ninth week. Signs of nerve lesions usually appeared between 6 and 9 weeks of age and developed rapidly.

In general, the paralysis appears at about the time the rate of weight gain begins to fall below the normal, which is about one week before a decrease in weight begins. In many rats paralysis developed while weight was still increasing

21. Swank, R. L., and Davenport, H. A.: Stain Technol. **9**:11, 1934.

22. Not only were no degenerative changes in the myelin found but the diffuse blackening of occasional fibers mentioned by Swank (J. Exper. Med. **71**:683, 1940) as a feature of prolonged malnutrition in rats was absent. In later experiments in rats examined by Dr. Swank, the same degree of blackening of myelin sheaths was found in rats with prolonged vitamin A deficiency but no neurologic disturbances as in their starvation controls. The significance of the "starvation effect" in myelin requires further study and confirmation of the premises.

at a normal rate and as much as two weeks before the deficiency could be detected by a decrease in weight or other gross signs. Rats in which marked loss of weight occurred early as a result of inanition, before nervous signs appeared, died with all the usual epithelial lesions of vitamin A deficiency but without the signs or lesions of nerve degeneration.

The first sign is incoordination of the hindlegs, evidenced by an unsteady and uneven gait and by occasional overabduction or overadduction of one or both legs. One or both hindlegs may be affected, but the two legs may vary in incapacity of function. The disorder may not progress beyond ataxia and incoordination, especially if the onset was beyond the eighth week and the rat over 100 Gm. in weight. On the other hand, with early onset of signs, the disorder progresses to complete paralysis or inability to use the hindlegs.

Paralysis usually is present before the eighth week of life. If it appears after this age, it is less frequent and less severe. It has occurred in a significant number of rats up to the twelfth week but very rarely beyond this age (fig. 1). Rats which grow to weigh over 125 Gm., regardless of age, rarely show paralysis, although all the other signs of vitamin A deficiency develop.

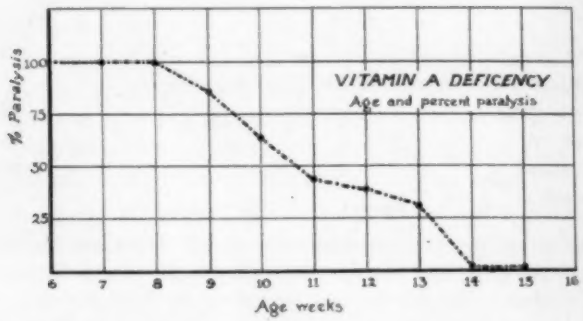

Fig. 1.—The chart presents graphically the frequency of occurrence of paralysis in 300 rats in relation to the age at which the deficiency was established as indicated by loss of weight. For example, the graph indicates that if the deficiency was established by the end of the eighth week, 100 per cent of the animals became paralyzed, whereas if it was established at the age of 10½ weeks, paralysis developed in only 50 per cent of the rats.

One very important fact, to be discussed more fully later, is that the onset of signs and the development of the nerve lesions are rapid and are established before the rats cease to gain weight. The onset of nervous signs can be prevented if the administration of vitamin A in some form is begun seven to ten days before they are expected to appear. Administration of vitamin A begun at 42 days of age always prevents paralysis and often does if begun as late as 47 days of age. Paralysis is usually apparent by the fifty-fourth day of age.

Animals whose deficiency is cured by the administration of vitamin A in some form after the development of paralysis remain permanently crippled, although they are restored to health in all other respects.

Administration of alpha tocopherol (vitamin E) does not prevent the development of the nerve lesions.

PATHOLOGIC OBSERVATIONS

The fact that the consistent production of nerve disorders in rats in consequence of vitamin A deficiency required the establishment of the deficiency at an early period of growth made the mere demonstration of myelin sheath degeneration in the peripheral and central nervous systems unsatisfactory unless there was correlation with some aspect of physiology or growth.

Routinely, myelin sheath degeneration was found in the sciatic and brachial nerves, spinal cord, medulla and posterior cerebellar peduncles. The trigeminal nerve very rarely showed a few degenerated fibers. Studies of the spinal cords, medullas and midbrains from 13 paralyzed rats confirmed, in general, the distribution of lesions as given by Mellanby [3d] for the rabbit, and Zimmerman [7] and Irving and Richards [12] for the rat.

Two factors came to light which were against the probability of finding a correlation either with the physiology of the nervous system or with its postnatal development. These were, for the first, the too great irregularity in distribution and in degree of involvement of the tracts in the spinal cord as shown by myelin sheath degeneration and, for the second, the lack of relation of the tracts most constantly involved to the order of postnatal myelination in the spinal cord of the rat as described by Watson [23] and Buckley.[24]

Differences in degree and in distribution of myelin degeneration in the spinal cord were marked when the preparations from different rats were compared. In individual rats there were often marked differences in the two sides of the spinal cord; occasionally tracts which showed many degenerated fibers on one side would be practically free from lesions on the opposite side.

In regard to postnatal development, the regions most regularly and most conspicuously degenerated in the vitamin A–deficient rats were those roughly described by Zimmerman [7] as the sensory tracts on the periphery of the spinal cord, and these [25] are among the very first to become myelinated. The posterior columns, which frequently contain many degenerated fibers, also are among the earliest to undergo myelination. As there are no myelinated fibers in the nervous system of the rat at birth [25] and myelination is still incomplete at 42 days of age, our observations seemed incompatible with the idea that vitamin A–deficiency directly affects maintenance or formation of myelin. If vitamin A were necessary for formation or maintenance of myelin, fibers

23. Watson, J. B.: Animal Education: An Experimental Study on the Psychial Development of the White Rat Correlated with the Growth of Its Nervous System, Chicago, University of Chicago Press, 1903.

24. Buckley, A. C.: J. Comp. Neurol. **66**:449, 1937.

25. Watson.[23] Buckley.[24]

undergoing myelination during the age period of the experiments should have exhibited the severest consequences of the deficiency.

Gross anatomic studies restricted to dried preparations of skeletons were unproductive. No significant differences were found in the exteriors of skeletons of paralyzed rats as compared with several types of controls. Serial sections of the lumbar and upper sacral vertebrae with spinal cord and nerves and adjacent soft tissues in situ were made of a few severely paralyzed rats, cut in the horizontal (frontal) plane and in cross section. These sections showed great distortions of the spinal ganglions and nerve roots and deep pits occupied by coiled nerve roots in the bodies of the lower lumbar and upper sacral vertebrae (fig. 2). Further progress in elucidation of the alterations was made by gross dissection of the central nervous system in situ.

The following description is based on the dissection of 25 vitamin A–deficient rats which had shown all stages of paralysis from the earliest signs to maximum severity.[26]

The appearance of the exposed nervous system of a markedly paralyzed rat is that of overcrowding—of a central nervous system too large for its bony encasement—with most striking evidences in the skull and in lumbar and sacral vertebrae.

The prominent features in the skull are a herniation of the cerebellum into the foramen magnum and multiple herniations of the cerebrum and cerebellum into the venous sinuses of the dura. In the spinal canal the prominent features are herniations of nerve roots into the intervertebral foramens and into the bodies of vertebrae and a low position of the conus medullaris.

The Brain.—The following description is a composite one, based on numerous examinations of rats which exhibited marked paralysis of seven to twenty-one days' duration and were killed at ages of from 56 to 77 days. Only the most apparent changes are described.

During exposure of the brain, thinning of the parietal and interparietal bones may be evident, and there may be loosening of the intervening sutures. The brain is flattened. The posterior border of the cerebellum presents as a deeply injected transverse band because of

26. The rats were prepared for dissection by evisceration and skinning, and fixation in solution of formaldehyde U. S. P. (1:10 D) after having been stretched on a board so as to keep the head and spine in as nearly straight a line as possible. After fixation the rats were decalcified in 5 per cent nitric acid, unless careful histologic studies were contemplated. Dissections were made under a low power, wide field binocular dissecting microscope and consisted of exposure of the brain, medulla and spinal cord by removal of bone from the dorsal side. Rarely, an approach was made from the ventral side in order to study the relations of the contents of pits in the vertebrae to the spinal ganglions. Dissections of undecalcified rats were not difficult, though they required more time.

molding of the posterior lobe into the foramen magnum (fig. 4 *A*). Herniations of the cerebellum and cerebrum are usually conspicuous in the transverse sinuses and in the lacunas which receive the superior

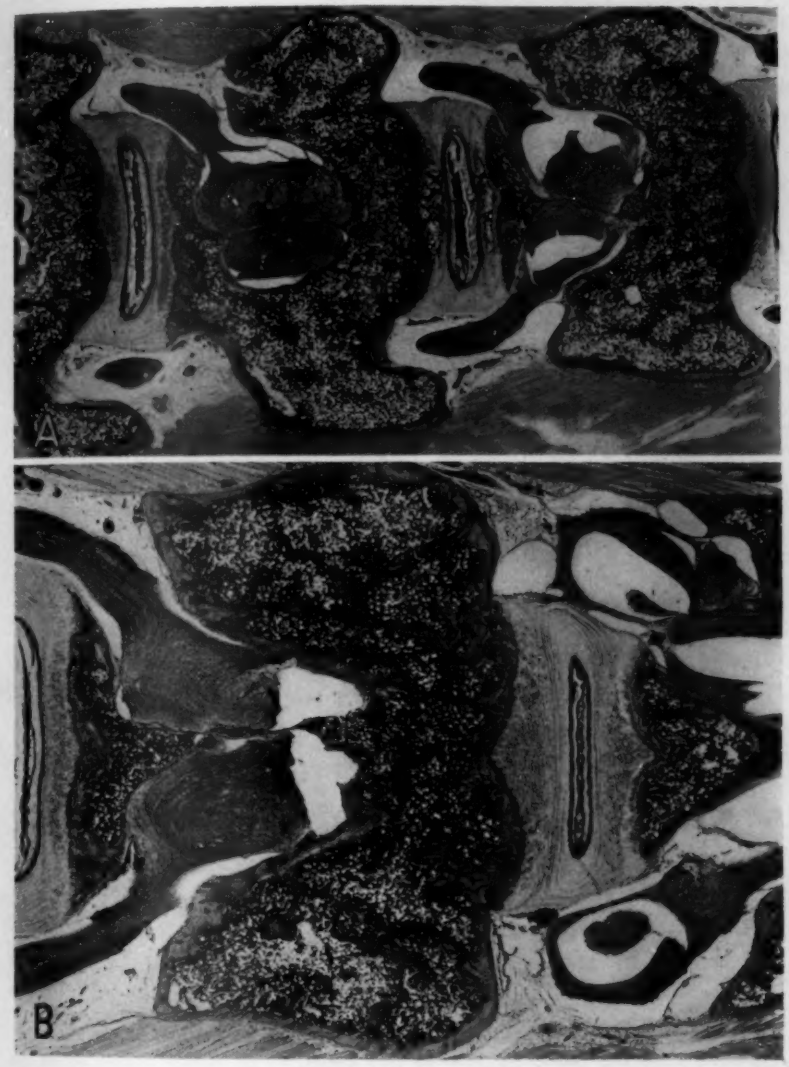

Fig. 2.—*A*, photomicrograph at 9 diameters; a vitamin A–deficient rat 65 days old, which had shown paralysis for nine days. The horizontal section shows, from right to left, the third and the fourth and a part of the fifth lumbar vertebra, to illustrate pitting of vertebrae.

B, photomicrograph at 15.5 diameters; a vitamin A–deficient rat 65 days old, which had shown paralysis for eight days. The horizontal section shows, from right to left, a part of the third and the whole of the fourth lumbar vertebra, to illustrate pitting of vertebrae.

cerebellar veins. The common sites of origin of the hernias are: for the posterior poles of the cerebral hemisphere, (1) the junction of superior petrosal and transverse sinuses and (2) the points where superficial veins of the cerebrum enter the transverse sinuses; for the cerebellum, (1) the junction of the superior cerebellar veins and the lacunas of the transverse sinuses into which they empty, (2) the angles at the junctions of the aforementioned lacunas and the transverse sinuses and (3) the junctions of the inferior cerebellar veins and the transverse

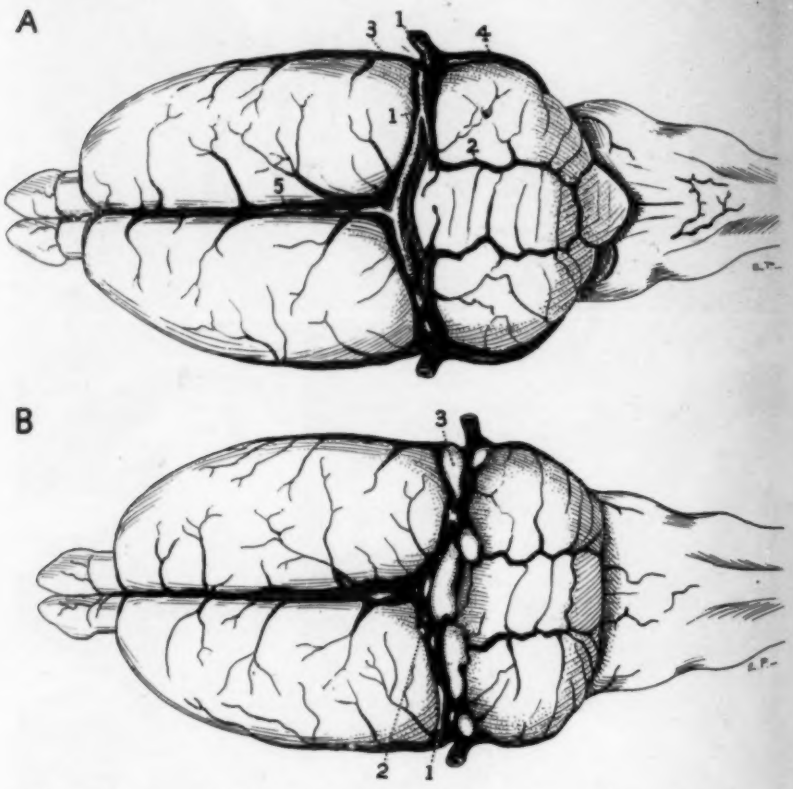

Fig. 3.—*A*, normal brain showing dural sinuses and cerebellar veins: *1*, transverse sinus; *2*, superior cerebellar vein; *3*, superior petrosal sinus; *4*, inferior cerebellar vein; *5*, superior sagittal sinus.

Note the lacunas of the transverse sinuses where the superior cerebellar veins enter. We have not found mention of this detail. The region seems to be an important one for the drainage of cerebrospinal liquid because of the abundance of arachnoidal villi.

B, composite drawing of herniations into the dural sinuses. *1*, *2* and *3* are herniations of the posterior poles of the cerebrum. *1* and *3* have entered at the junctions of the superior petrosal and transverse sinuses. The other herniations are of the cerebellum. Compare with the drawing of the vessels of the normal brain for points of entrance. Compare also with figure 4 *A*.

sinuses. In some instances there was a herniation of the cerebellum into the torcular, presumably at the entrance of the straight sinus (figs. 3 and 4 A).

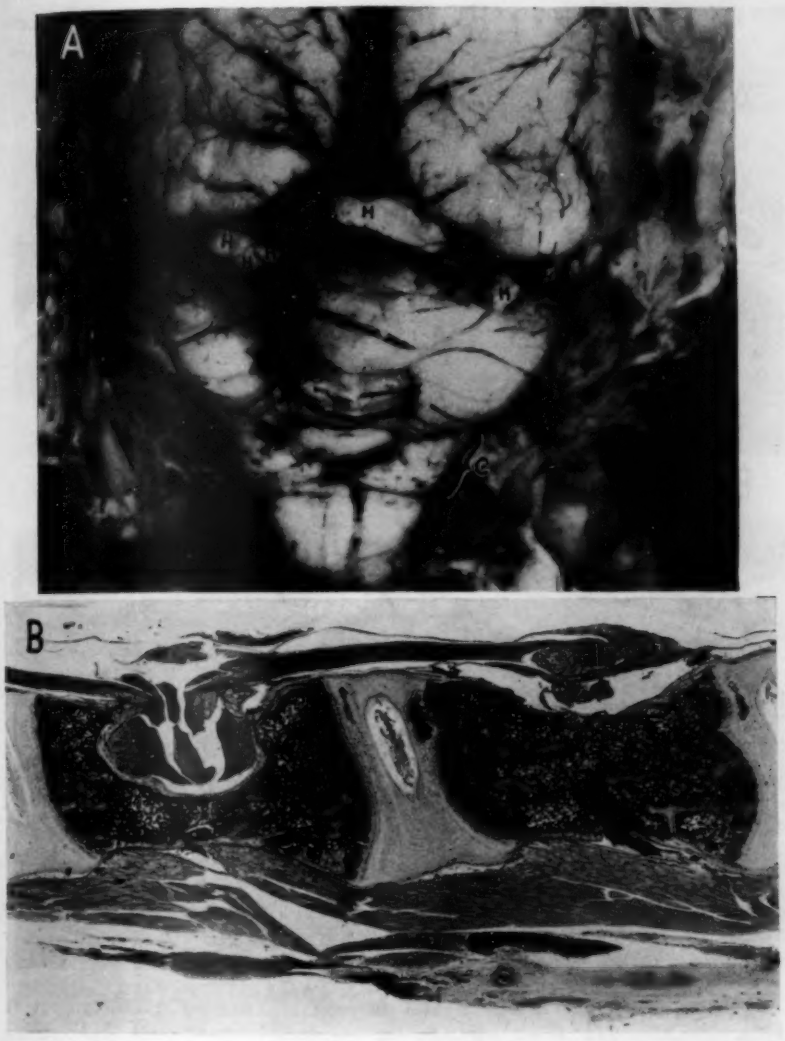

Fig. 4.—*A*, photograph at 5 diameters; brain of a vitamin A–deficient rat 9 weeks old, which had shown slight paralysis for sixteen days. Note the herniations (*H*) of cerebrum and cerebellum into the transverse sinuses and torcular and the flattening of the posterior lobe of the cerebellum where it was forced into the foramen magnum (bracket at *C*). Compare with figure 3 *B*.

B, photomicrograph at 10.7 diameters; a vitamin A–deficient rat 8 weeks old, which had shown paralysis for seven days. The sagittal plane section shows, from left to right, the fifth and sixth lumbar vertebrae.

In all of the aforementioned sites, structures corresponding to the arachnoidal villi of man have been found in normal rats by means of serial sections, made through these regions, of decalcified heads of normal rats. Arachnoidal villi, substantially identical with those of man, are present in relation to the venous sinuses and to the larger super-

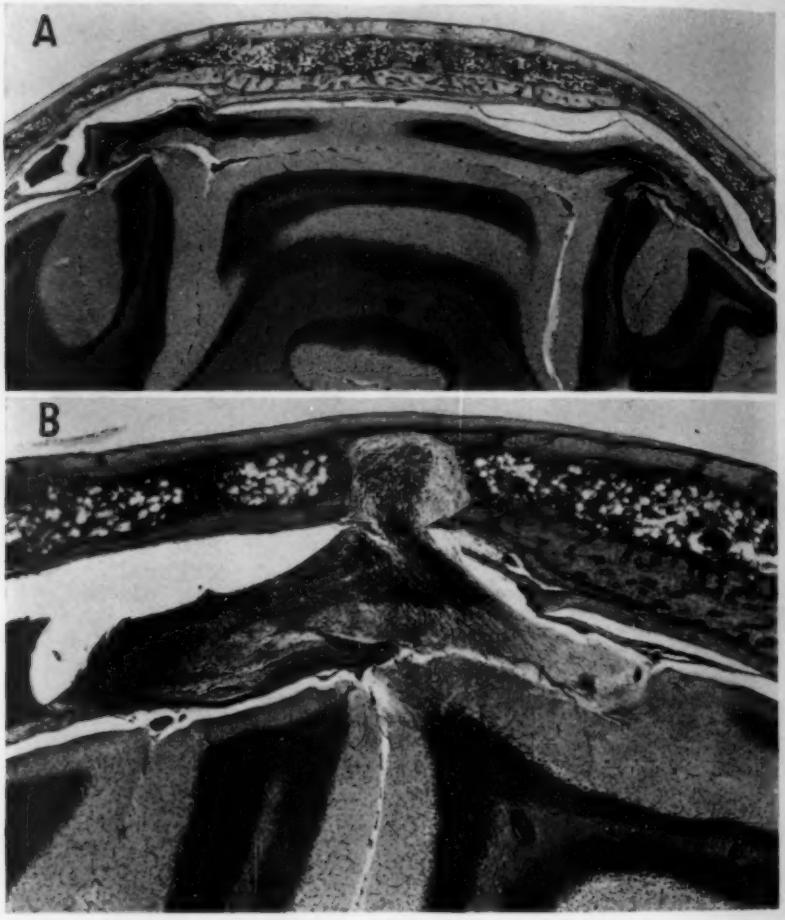

Fig. 5.—*A*, photomicrograph at 14.3 diameters; a vitamin A–deficient rat 10 weeks old which had shown paralysis for six days. The frontal plane section through skull and cerebellum shows a herniation on each side into the lacuna where the cerebellar vein enters the transverse sinus.

B, photomicrograph at 35 diameters from the same rat as *A* but at a different level, showing a pit in interparietal bone occupied by herniated cerebellar tissue. The increased thickness of the inner table is due to the proximity to the sutures with the parietal bones, but the new bone formation is somewhat greater than in normal rats.

ficial cerebral and cerebellar veins. At the junctions of the superior and inferior cerebellar veins with the transverse sinuses and at the junctions of superior petrosal and transverse sinuses, and at the confluence of the sinuses, there are projecting extensions of the arachnoid membrane structurally composed like human arachnoidal villi, usually sessile, less commonly pedunculated, and having the same relation to the blood stream.

The largest of these arachnoidal villi range in size—i. e., diameter at base—from 0.1 to 0.6 mm. They are most prominent in the angles of junctures of the superior cerebellar lacunas and transverse sinuses. The mechanism of herniation into arachnoidal villi in these rats is probably the same as that for the multiple herniations of the cerebrum and cerebellum into arachnoidal villi in man in consequence of increased intracranial pressure.[27]

The herniations may reach considerable size and may extend for a distance of 2 to 3.5 mm. into the transverse sinuses (figs. 5 *A* and 6 *A*). In a rat studied by serial sections made through brain and skull there were found (fig. 5 *B*) extensions of herniated cerebellar substance into the interparietal bone with pit formations extending to the outer table, presumably taking origin at points of entrance of emissary veins.

The few herniations of the cerebellum which have been studied histologically were capped by arachnoidal tissue (fig. 6 *B*) indicative of their sites of entrance and similar to findings in human herniations of similar genesis.[27]

Other distortions of the brain were found. Among the most conspicuous were: (1) incarceration and compression of the paraflocculi, the result of new bone formation in their fossae; (2) anteroposterior flattening and dorsal extrusion of the posterior colliculus; (3) dilatation of the cerebral ventricles (not constant), and (4) flattening and tortuosities of the optic nerves.

The new bone formation in the fossae housing the paraflocculi is of periosteal origin and is restricted to those parts of the fossae which are in direct relation to the bony labyrinths of the ears (figs. 7 *B* and 8). It is similar in structure to the periosteal bone formation described by Mellanby [4] in the internal auditory meatus of vitamin A–deficient puppies (fig. 7 *A*) and by Moore and associates [28] in the optic foramens of vitamin A–deficient calves.

The Spinal Cord and Nerve Roots.—There is no empty space in the spinal canal of the paralyzed vitamin A–deficient rat.[29] The dura is

27. Wolbach, S. B.: J. M. Research **19**:153, 1908.

28. Moore and associates.[13] Moore.[14]

29. Cerebrospinal fluid relations have not been investigated. Appearances in celloidin-embedded spinal columns (fig. 2) suggest that cerebrospinal fluid may be entrapped in herniations into intervertebral foramens. However, great allowance must be made for shrinkage in decalcified and celloidin-embedded nerve tissues.

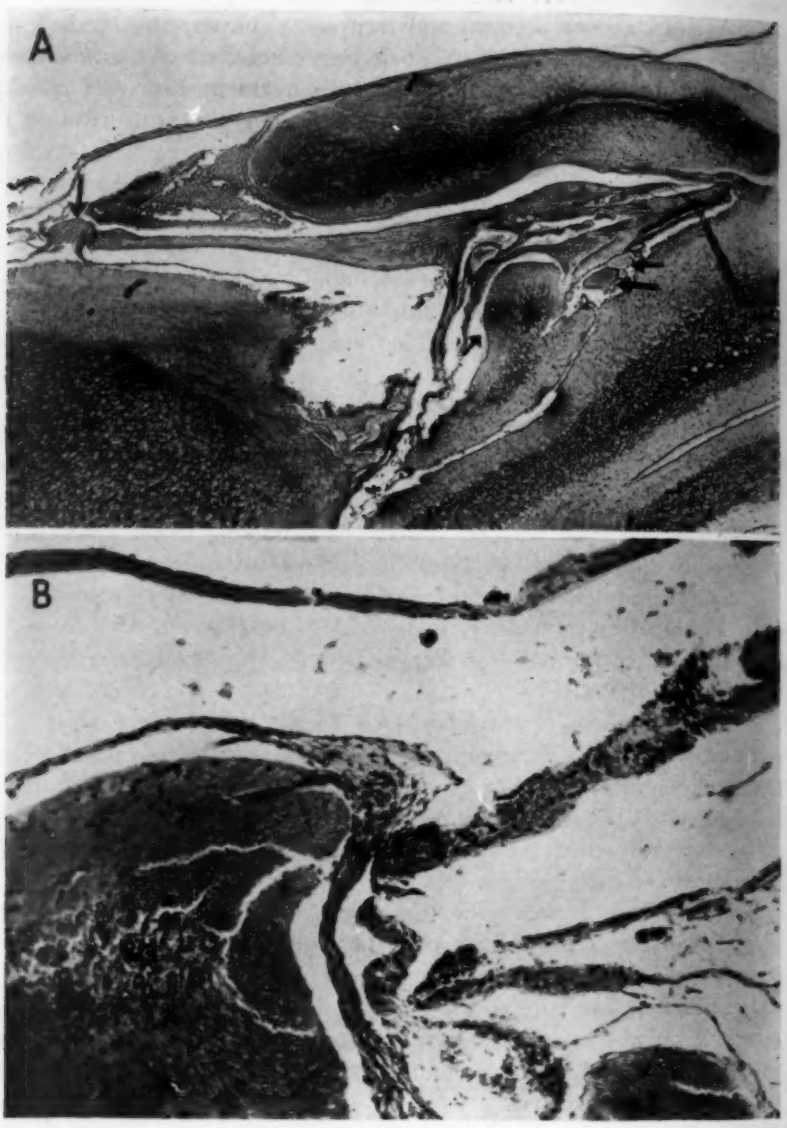

Fig. 6.—*A,* photomicrograph at 35 diameters; a vitamin A–deficient rat 60 days old, which had shown severe paralysis for eight days. This is a Nonidez-Cajal silver preparation to show one large and several small herniations of the cerebellum.

B, photomicrograph at 116 diameters; a vitamin A–deficient rat 60 days old, which had shown severe paralysis for eight days; hematoxylin and eosin. Note the herniation of the cerebellum into the transverse sinus. Note the arachnoidal villus cap.

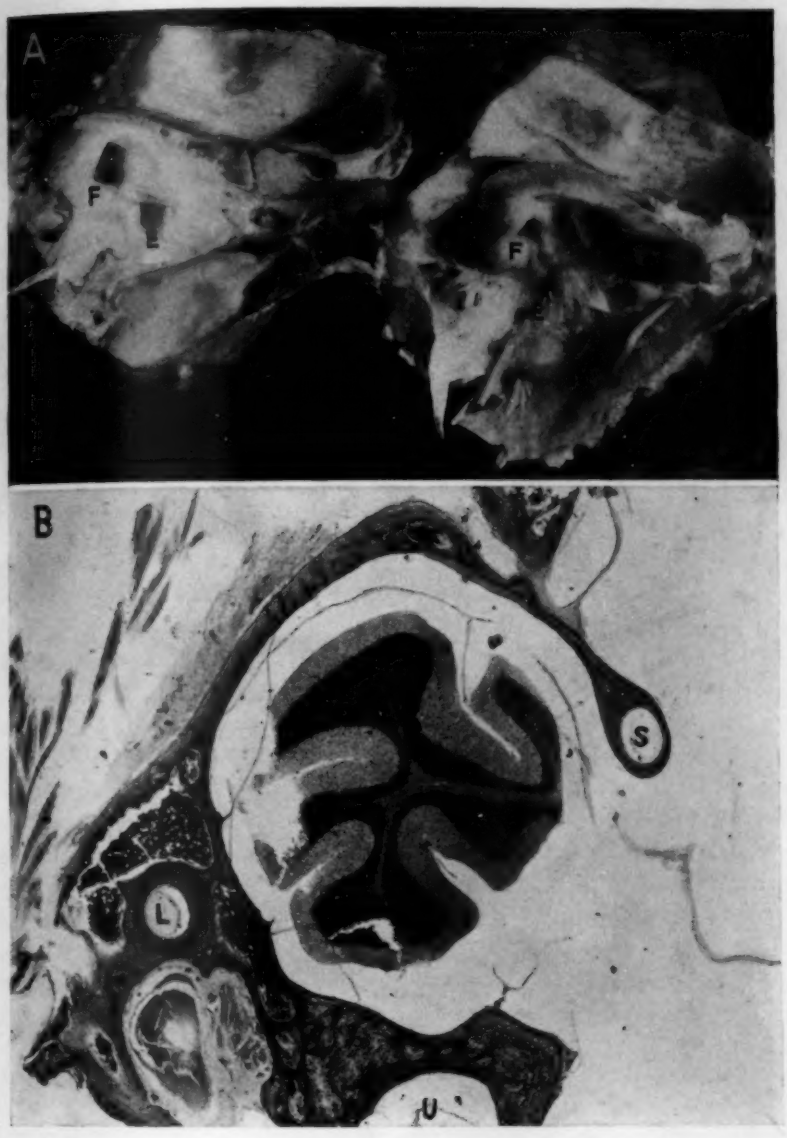

Fig. 7.—*A*, photographs at 1.6 diameters of the left petrous bones of litter mate puppies. That on the left is from the normal control. That on the right is from the puppy deficient in vitamin A. *F*, indicates the fossa for the paraflocculus; *E*, the internal auditory meatus. Note the general overgrowth of bone and the effect on *F* and *E*.

B, photomicrograph at 25 diameters; a normal control rat 54 days old; section through fossa of the right paraflocculus. *S* indicates the superior canal; *L*, the lateral canal, and *U*, the utricle.

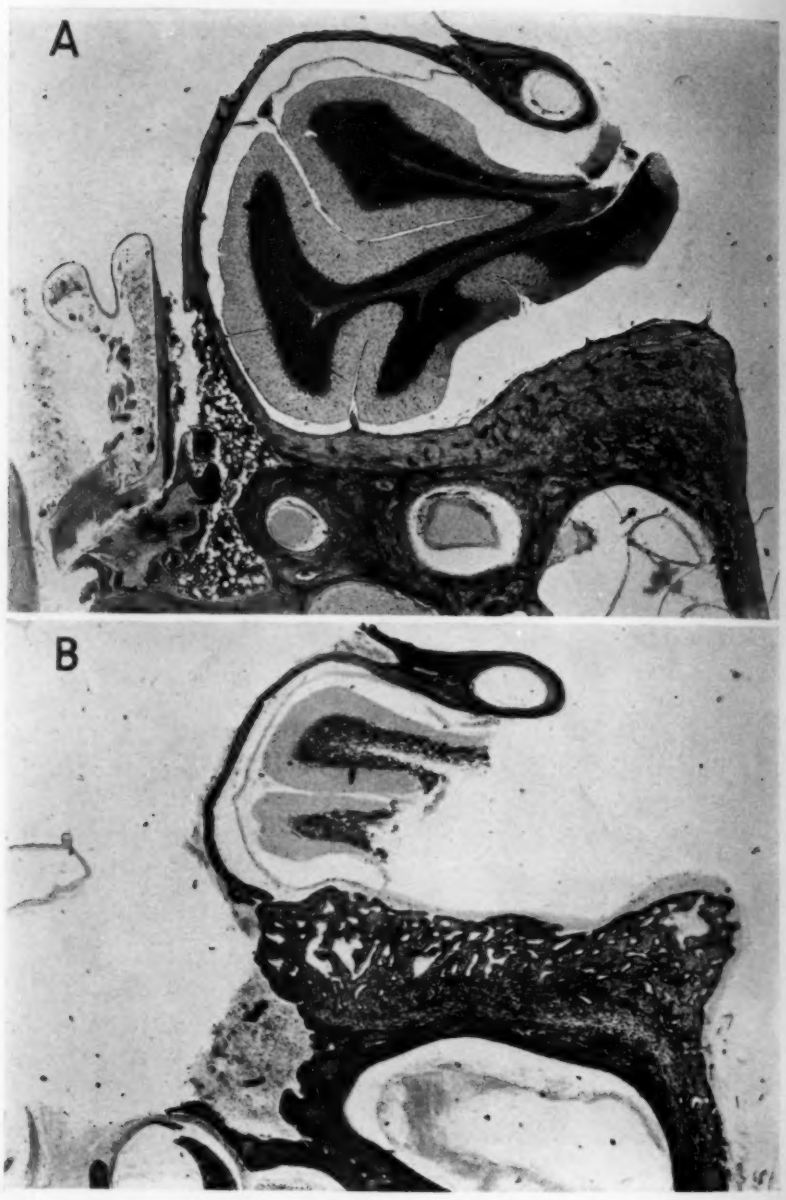

Fig. 8.—*A*, photomicrograph at 26.3 diameters; a vitamin A–deficient rat 61 days old, which had shown paralysis for ten days; section through the fossa of the right paraflocculus.

B, photomicrograph at 26.3 diameters; a vitamin A–deficient rat 68 days old, which had shown paralysis for fifteen days; section through the fossa of the right paraflocculus.

tightly applied to the spinal cord and cauda equina. The posterior root ganglions of the lumbar and sacral regions are apparently enlarged, but after the dura has been opened, this apparent increase in size of the ganglions is seen to be due to extrusion of one to five or six flexed nerve roots (dorsal and ventral) into the intervertebral foramens (fig. 9). The ganglions are displaced laterally and are indented or invaginated by the herniated nerve roots (figs. 10, 11 and 12). The dura is outpocketed into the ganglions and tightly adherent thereto. Nerve roots from one side may cross the median line and be herniated into foramens of the opposite side. Rarely, and only in instances of long-continued severe paralysis, the lumbar cord is molded (herniated) in one or two places into intervertebral foramens or into pits containing nerve root herniations in the bodies of vertebrae (fig. 12).

The most striking manifestation of the disparity in size of the spinal canal and that of its contents is the bilaterally symmetric pitting of the bodies of the lower lumbar and upper sacral vertebrae (fig. 2). These pits contain herniations of the nerve roots which lie on the ventral and lateral sides of the spinal cord and usually contain also the flexed or coiled ends of anterior nerve roots before they take exit in their respective foramens. These pits reach a large size, as may be seen in figures 4 *B* and 13 *B*. The locations of the pits are, in our opinion, determined by the presence of large veins on the ventral side of the spinal canal which receive tributaries from the bodies of the vertebrae and which communicate through the intervertebral foramens with veins outside the spinal column. Serial cross sections of normal and vitamin A–deficient rats indicate that it is the yielding of these compressible veins which first determines the sites of flexures of nerve roots when the spinal canal becomes too small to accommodate the relative overgrowth of the nervous system. The intervertebral foramens also are obviously regions of yielding to pressure. The pits in the vertebrae may be larger on one side than on the other. Each occupies an almost central portion of the vertebral body, a trifle caudad in some instances. In cases of pitting at a very early stage and in instances in which paralysis has been prevented by administration of carotene or vitamin A at 42 days of age, sections show clearly that the resorption of bone is in relation to the veins mentioned.

Study of sections of pitted vertebrae show osteoclasis of trabecular bone intersected by the pits. The coiled nerves are surrounded by connective tissue derived presumably from the dura and certainly from periosteum. There is always a fairly continuous zone of osteoblasts surrounding the pit contents, and always there are traces of osteoid, indicative of the fact that formation of bone is not wholly inhibited by the deficiency during the period of pit formation. Exposure of the

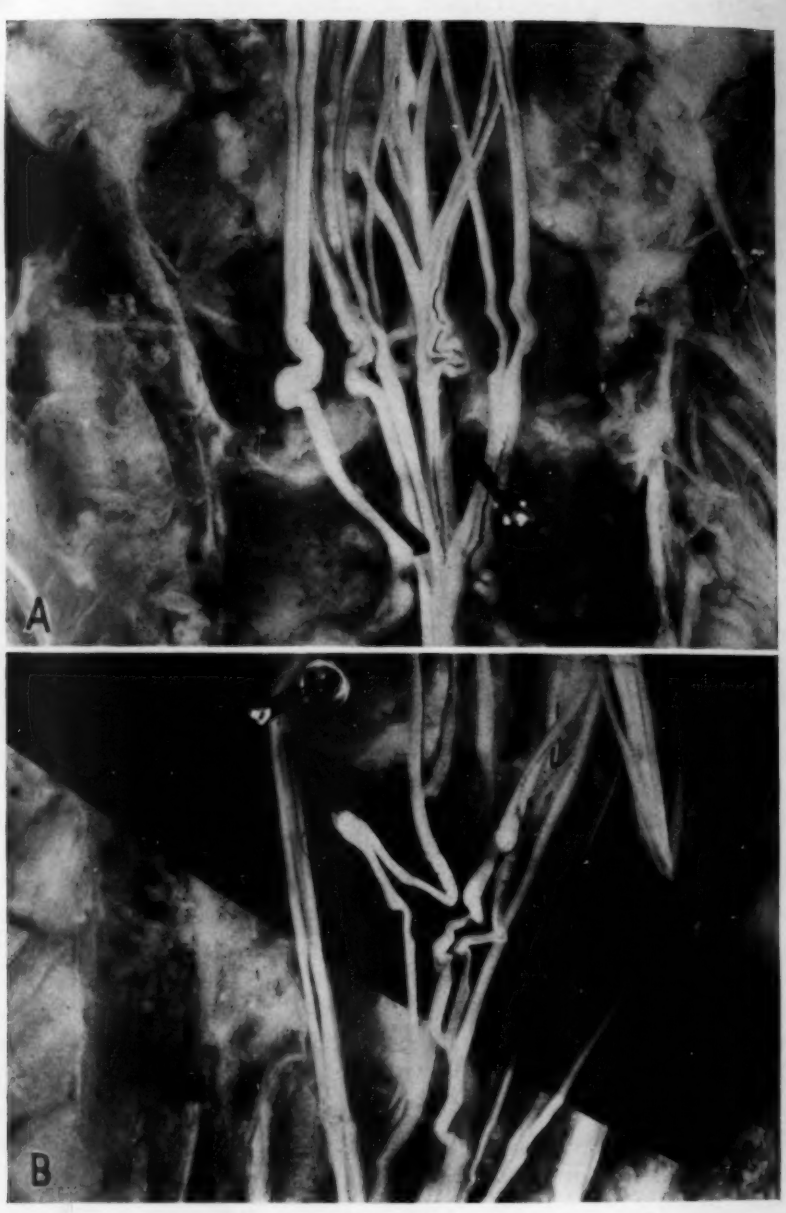

Fig. 9.—*A*, photograph at 7.4 diameters; a vitamin A–deficient rat 9 weeks old, which had shown slight paralysis for seven days. The dissection shows dislodged herniation of nerve roots at the level of the sixth lumbar ganglions and below a partial dissection at the level of the first sacral ganglions.

B, photographs at 7.6 diameters; a vitamin A–deficient rat 11 weeks old, which had shown slight paralysis for fourteen days. The dissection shows a few nerve roots dislodged at the level of the sixth lumbar ganglions.

herniations in the bodies of vertebrae from the ventral side by removal of bone is readily accomplished because of the investure by connective tissue structures. Removal of the latter reveals the coiled nerve roots. Figures 10 *C* and 13 *A* show better than words the coiling and distortion of the nerve roots and the size and relation of the pits.

A remarkable feature of this pitting of vertebrae of significance in the consideration of the growth factors concerned in this study is the rapidity of their formation. Many experiments, each with litter mate controls, have proved that the nervous manifestations and all accompaniment of anatomic changes, excepting in some instances the first discernible effects, are prevented by the administration of carotene or vitamin A at 42 days of age. Deep pits, however, have been found at 56 days of age with considerable regularity, as well as in all rats which showed paralysis before this time. These pits attain about maximum size after eight to twelve days of paralysis, representing ages of from 60 to 70 days.

Microscopic study of vertebrae of paralyzed rats which have been treated with vitamin A shows complete walling-off of the pits with newly formed bone and also bone formation between nerve roots within the pits, conforming with the usual sequences and distribution of reparative bone production.

It does not seem worth while to describe in minute detail all the appearances and variations in distribution of the nerve root herniations. The degree of herniation must obviously depend on the degree of disproportionate growth. Degrees of asymmetry are probably due to chance and dependent on earlier initiation of herniation on one or the other side. An outstanding feature is that a single dorsal nerve root may herniate into several of the intervertebral foramens it passes between its origin and its foramen of exit (figs. 10 and 11). Dorsal roots of the second and third sacral ganglions have been found with four herniations above their foramens of exit. Likewise, ventral nerve roots may herniate into several pitted vertebrae and also into one or more intervertebral foramens as chance flexions determine. Anatomic relations determine the relative position of nerve roots in a given herniation (fig. 10 *B*); the outermost roots in a given herniation have the highest attachment to the spinal cord. In a preliminary report [30] we mentioned small excrescences seen in the course of nerve roots and suggested that they might be comparable to amputation neuroma. These excrescences are found in relation to sites of herniation of other nerve roots; they are also found presenting anterior and posterior to spinal ganglions and taking origin both from ventral and from dorsal nerve roots. It is perhaps permissible to refer to them as herniations of the excrescence type because

30. Wolbach, S. B., and Bessey, O. A.: Science **91**:599, 1940.

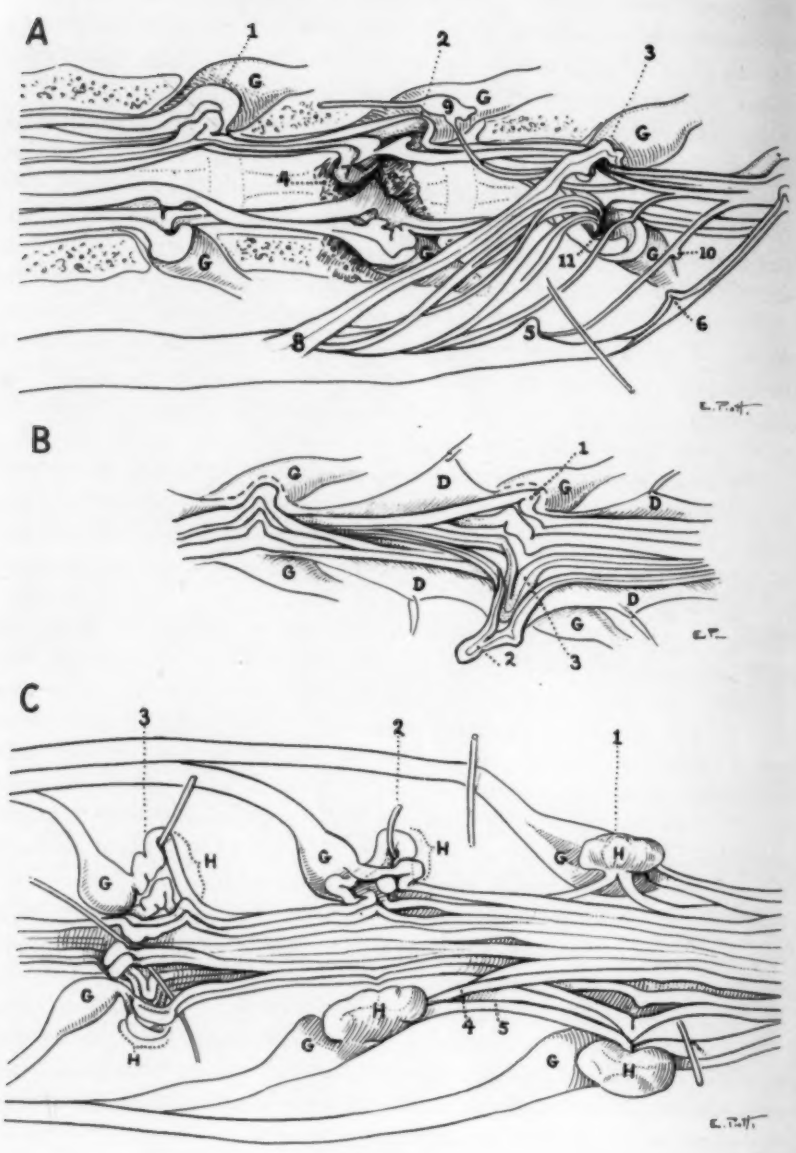

Figure 10

(See legend on opposite page)

they are produced by the buckling of degenerated nerve fibers in the direction of least resistance; more deeply situated nerve fibers in the trunks do swerve toward the excrescence, but there is no flexion of the nerve root as a whole.

Some degree of the disproportionate growth can be found in all nerve roots. In the upper dorsal and cervical roots no herniations have

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EXPLANATION OF FIGURE 10

A, vitamin A–deficient rat 10 weeks old, which had shown severe paralysis for nine days; dorsal dissection exposing third, fourth, and fifth lumbar ganglions, indicated by *1*, *2* and *3* respectively.

4 indicates where the body of the fourth lumbar vertebra has been dissected away, exposing coiled nerve roots which have pitted the bone.

5, herniation of the spinal cord, which was extruded into the right fourth lumbar ganglion.

6, herniation of nerve roots removed from the fifth lumbar ganglion.

7, ventral root of the fourth lumbar ganglion lying in a pit in the body of the fourth lumbar vertebra.

8, dorsal roots to the sixth lumbar and first sacral ganglions from their origin to herniation into the fifth lumbar ganglion.

9, herniation of a coiled and swollen unidentified nerve root removed from the invaginated fourth lumbar ganglion.

10, small excrescence on the ventral nerve root removed from a fifth lumbar ganglion.

11, a deep pit in the fifth lumbar vertebra with entering and emerging nerve roots.

G, ganglions.

B, vitamin A–deficient rat 11 weeks old, which had shown severe paralysis for twenty-one days; dorsal dissection exposing fifth and sixth lumbar ganglions with herniated nerve roots.

1, ventral root of the first sacral ganglion herniated into the sixth lumbar, left side.

2, dorsal and ventral roots of the third sacral ganglion, right, removed from herniation into the sixth lumbar ganglion, right.

3, dorsal and ventral roots to the second right sacral ganglion dislodged from herniation into the right sixth lumbar ganglion.

G, ganglions.

D, dura. Its line of attachment to the ganglion is represented by dotted lines.

C, vitamin A–deficient rat 10 weeks old, which had shown severe paralysis for nine days; ventral dissection. The bone was dissected away from the herniations occupying pits in the bodies of the fourth, fifth, and sixth lumbar vertebrae, indicated respectively by *1*, *2* and *3*. *H* indicates herniation; *G*, ganglion. The herniations in relation to the fourth lumbar ganglions are covered by membranes. Partial dissections have been made elsewhere to give an idea of the complexity of the coils and the irregular thickening of the herniated nerve trunks. *4* and *5* indicate dorsal and ventral roots of the left fifth lumbar ganglion entering the hernial sac.

Fig. 11.—A photograph at 7.3 diameters of the preparation from which figure 10 *A* was drawn; a vitamin A–deficient rat 10 weeks old, which had shown severe paralysis for nine days. The numerals *3, 4, 5* and *6* are placed over the third, fourth, fifth and sixth lumbar ganglions respectively. At *7* the bone of the fourth lumbar vertebra has been removed and shows the ventral root of the fourth lumbar ganglion within a pit. At *5* are many nerve roots entering and leaving pits in the fifth lumbar vertebra.

D indicates dura.

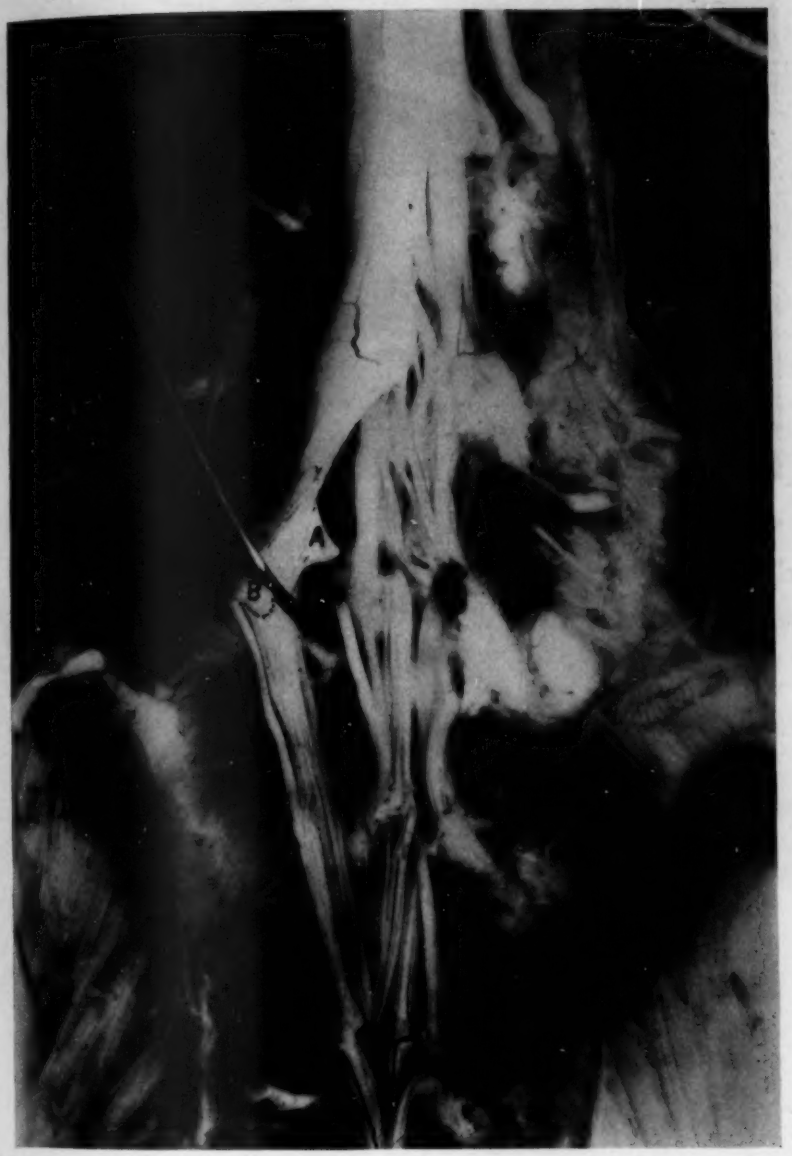

Fig. 12.—A photograph at 7.3 diameters; a vitamin A–deficient rat 10 weeks old, which had shown severe paralysis for fourteen days. The photograph shows molding and herniations of the spinal cord: one on the left, at *A*, into a pit in the fourth lumbar vertebra, and one on the right, at *B*, into the intervertebral foramen.

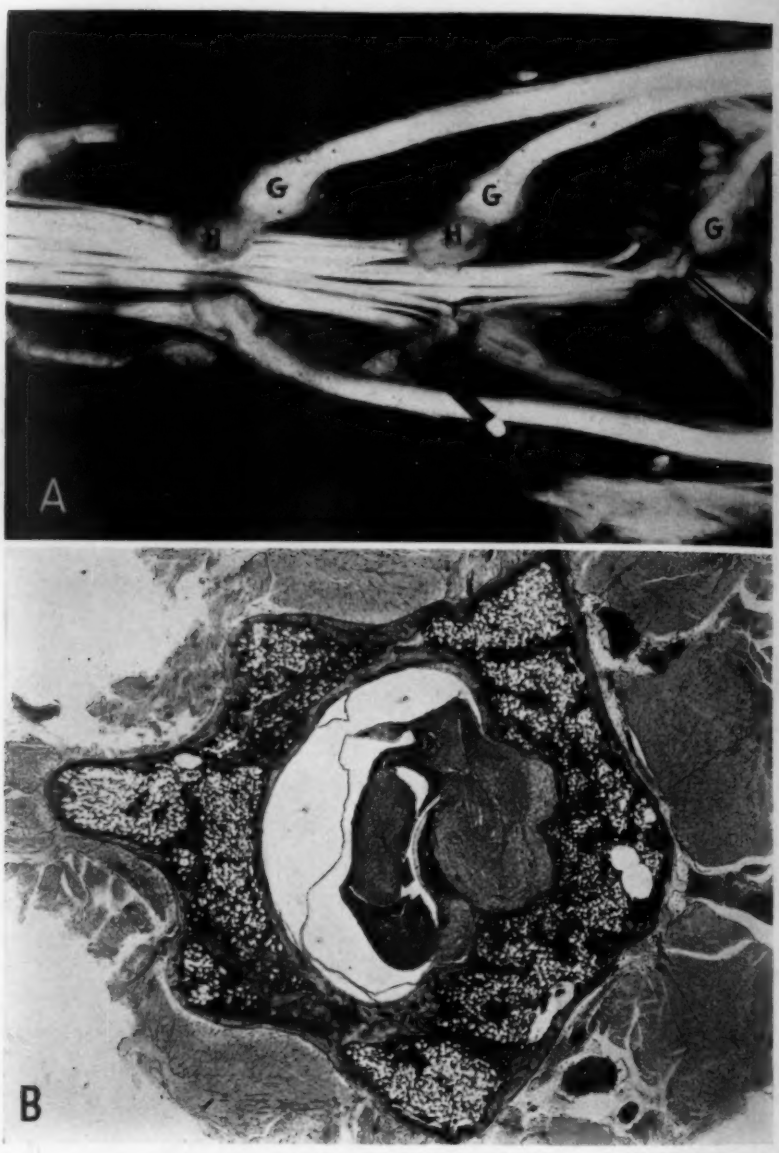

Fig. 13.—*A*, photograph at 6.5 diameters of the preparation from which figure 10 *C* was drawn; a vitamin A–deficient rat 10 weeks old, which had shown severe paralysis for nine days; ventral dissection at the levels of the fourth, fifth and sixth lumbar ganglions.

H indicates herniations; *G*, ganglions.

B, photomicrograph at 16.2 diameters; a vitamin A–deficient rat 9 weeks old, which had shown paralysis for eight to ten days; cross section of a lumbar vertebra showing pits occupied by nerve roots. The shrinkage due to embedding in celloidin gives the impression of much free space within the spinal canal. The cerebrospinal fluid relations have not been worked out in these vitamin A–deficient rats.

been found, but in rats exhibiting the most marked effects very definite bowing has been present. The degree of relative overgrowth of nerve roots increases from cervical to sacral regions and is maximal in lower dorsal and upper sacral roots.[31]

The length of the spinal cord from the posterior end of the fourth ventricle (calamus scriptorius) to the tip of the conus medullaris was measured in each dissected rat and the position of the tip of the conus medullaris recorded. Although in rats with vitamin A deficiency the compression of structures makes the determination of the exact position of the tip of the conus medullaris difficult and often uncertain, the difference between the normal position of this structure and that in the paralyzed rat is so great that errors of 1 or 2 mm. in locating it lose significance.

In normal rats and in rats severely stunted at an early age by being fed inadequate amounts of a complete ration and by riboflavin deficiency, the position of the tip of the conus medullaris throughout the age period of our experiments is approximately opposite the anterior (cephalad) end of the fifth lumbar vertebra. The variation is not much over 1 mm. in either direction. In the paralyzed rats with brain and nerve root herniations, the tip of the conus medullaris at 8 weeks of age is approximately opposite the anterior end of the sixth lumbar vertebra, and at 9 weeks of age or older it may be as low as the posterior (caudad) border of the sixth lumbar vertebra.

Observations of a small series of normally growing rats between 40 and 60 days of age give 5 mm. as the increase in length of the spinal cord each week. The average length of lumbar vertebrae at 56 to 63 days (taken from roentgen films) is very close to 5 mm.

The relative overgrowth of the spinal cord in vitamin A deficiency at 8 to 9 weeks of age is therefore approximately the length of one lumbar vertebra, a relative increase representing growth of at least one week.

The origins of the dorsal nerve roots of the lower lumbar and sacral ganglions are lower than the corresponding normal roots by distances of one fourth to three quarters of the length of a lumbar vertebra. Therefore, portions of a given nerve root totaling a length of 6 to 8 mm. may be involved in its numerous herniations.

Histologic Features of the Herniations.—The herniations of the cerebrum and cerebellum into the sinuses consist of cortical and medullary substance exhibiting various degrees of degeneration, necrosis and con-

31. Normally the growth of the spinal cord is proportionally less than that of the spinal column, so that the sacral end of the spinal canal moves away from the cord. The spinal nerve roots, therefore, elongate most markedly at the caudal end, and progressively less toward the head.

current cellular responses on the part of phagocytic cells and neuroglia. However, surprisingly well preserved cerebellar cortex and many apparently viable Purkinje cells are present in herniations of large size. Extensive loss of nerve fibers is apparent in preparations made by the use of Nonidez' [32] modification of Cajal's technic. No evidence of regeneration of axons could be found. The herniations into the venous sinuses are covered by pia-arachnoid and by endothelium derived from the blood vessel. The extremities of the herniations are capped with loose-textured arachnoidal tissue (fig. 6*B*) derived from the arachnoidal villi of the rat we have described.

A description of the nerve root herniations would include all the classic features of wallerian degeneration and many of the phenomena of axon regeneration. Serial sections of lumbar and sacral spinal ganglions and the accompanying herniated nerve roots from three rats, 55 to 60 days old and paralyzed seven to thirteen days, have been studied by ordinary technics for nerve cells and by Nonidez' silver impregnation technic.

Degenerations of myelin sheaths of nerve trunk fibers are conspicuous even with ordinary stains. With eosin–methylene blue and cresyl violet stains, surprisingly little damage could be found in spinal ganglions in view of the marked gross distortion in lumbar and sacral levels; only occasional ganglion cells show degenerative changes; rarely a cell at the periphery of a ganglion, where subjected to greatest pressure, is completely degenerated.

Nerve roots where sharply flexed in hernations are swollen and vacuolated. Nerve fibers are vacuolated and filled with myelin globules of all sizes and shapes, and phagocytic cells are numerous. On the concave side of acutely flexed trunks there are great numbers of cells, presumably of sheath of Schwann origin, many in mitosis (fig. 14). In general, the classic details of degeneration and early reparative reactions following traumatic injury to nerve trunks are present.

In the sections of the silver-impregnated preparations, particularly in the proximal (central) arms of herniated loops of anterior nerve roots, all the details described by Cajal [33] in his classic studies of degeneration and regeneration of nerves following crushing injuries are present. Nerve fibers undergoing regeneration can be traced into areas filled with products and sequelae of myelin degeneration. Fibers with collateral and terminal branching are abundant, and at greater distances proximal or central from the regions of greatest myelin degeneration great numbers of nerve fibers show varicosities and lateral polypoid

32. Nonidez, J. F.: Am. J. Anat. **65**:361, 1939.

33. Ramón y Cajal, S.: Degeneration and Regeneration of the Nervous System, translated and edited by R. M. May, New York, Oxford University Press, 1928.

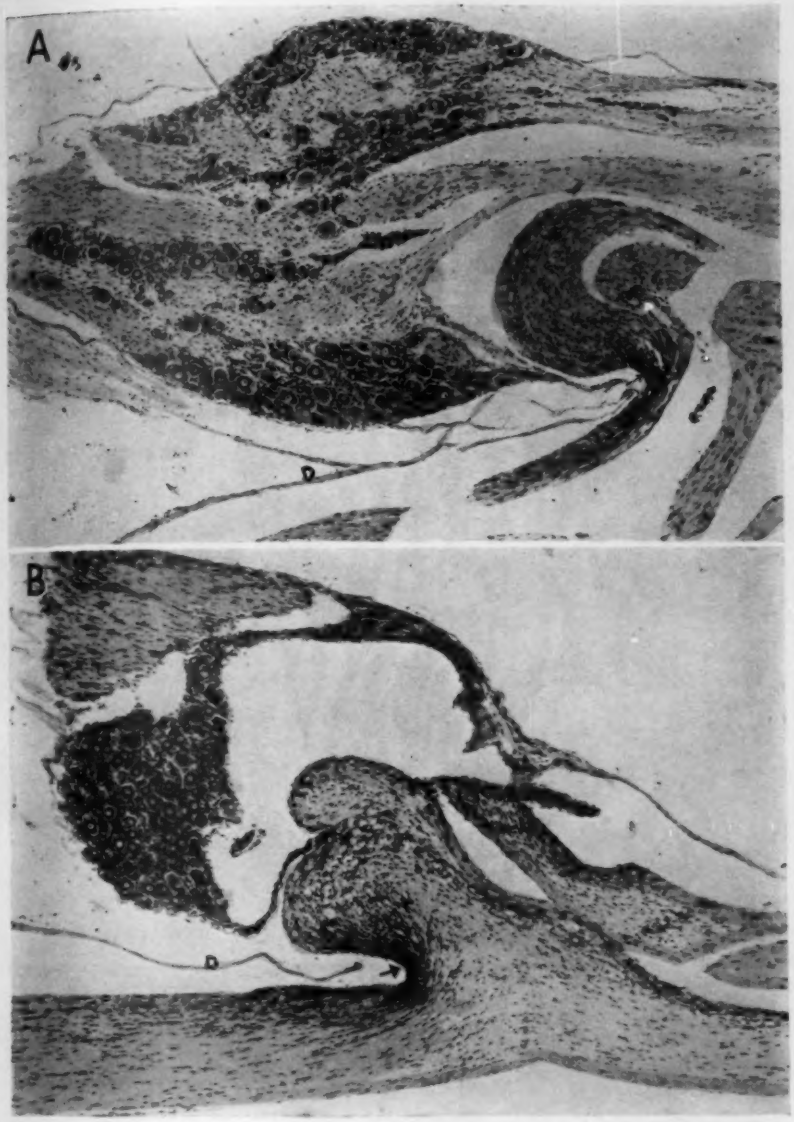

Fig. 14.—*A*, photomicrograph at 52.6 diameters; a vitamin A-deficient rat 56 days old, which had shown paralysis for six days; fifth lumbar ganglion; cresyl violet stain. The preparation shows the relation of the dura (*D*) to the hernia. Note the massing of nuclei of Schwann cell origin where the emerging arm of the herniated nerve root is flexed, indicated by the arrow. Note vacuoles among the nerve fibers.

B, photomicrograph at 52.6 diameters; fourth lumbar ganglion, same rat, same technic, as of *A*. A herniation of the excrescence type is shown. Note the increase of Schwann cell nuclei and the marked vacuolation among the degenerated nerve fibers.

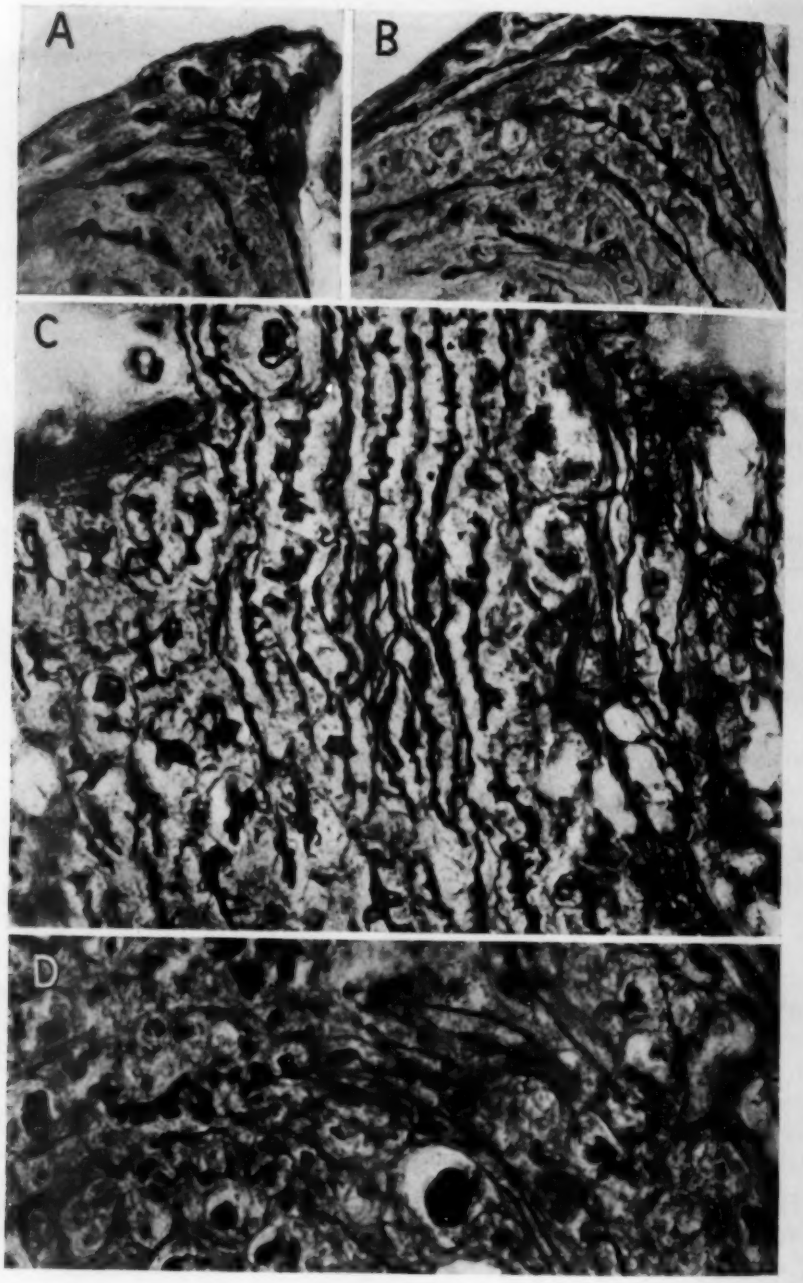

Figure 15

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sprouts, often reticulated. Huge or giant encapsulated clubs or spheres are very common, often reticulated, frequently degenerated or disintegrated. Fine terminal twigs are fairly numerous; rarely, they are found in bouquet form. Similar evidences of regeneration are seen in herniations of the excrescence type (fig. 15).

In the spinal ganglions similar, though less marked and apparently more recent, evidences of regeneration are present in the efferent (central) fibers near their points of exit. Few fibers in the ganglion itself show appearances of regeneration. Most of the fibers appear to be normal, as is true of the majority of fibers in the afferent nerve trunk. The motor nerve fibers outside the spinal canal at some levels are almost completely degenerated.

These few observations are recorded not because they are other than the expected consequences of injury to nerves produced by constriction but because they are proof that the axons in vitamin A deficiency have undiminished powers of regeneration. The appearances of many of our preparations approximate closely those of Cajal's for similar periods following injury if we rate the vitamin A deficiency lesion as beginning shortly before clinical signs appear.

COMMENT

Now that the genesis of the nerve lesions in vitamin A deficiency is clear, it is not profitable to discuss the diverse findings of various authors in regard to the neurons and tracts involved. The distribution of lesions is the consequence of pressure effects in the skull and spinal canal, and hence probably no type of neuron is exempt. We are certain that motor and sensory nerves and nerves of the special senses are

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EXPLANATION OF FIGURE 15

A and *B*, photomicrographs at 610.5 diameters; a vitamin A–deficient rat 60 days old, which had shown severe paralysis for six days; Nonidez-Cajal silver technic. This is a dorsal root from an undetermined level which was herniated into the second sacral ganglion. Both photomicrographs are from the same field but at different levels. They show various types of sprouts and new fiber formation indicative of regenerative processes.

C, photomicrograph at 610.5 diameters from the same rat as *A* and *B*; Nonidez-Cajal silver technic; herniation of the excrescence type from an anterior nerve root into the fourth lumbar ganglion. Note the many lateral sprouts, often reticulated, and the terminal reticulated spheres.

D, photomicrograph at 610.5 diameters; Nonidez-Cajal silver technic; a vitamin A–deficient rat 55 days old, which had shown severe paralysis for thirteen days; herniation of the excrescence type of a dorsal nerve root of undetermined source into the fourth lumbar ganglion. Note the large spheres, some reticulated, others degenerated, also fibers with lateral sprouts of various types.

involved. The account of the distribution of lesions in the rat given by Irving and Richards [12] is most in accord of any in relation to genesis.

The nature of the lesions described in the foregoing pages and other facts gained from a number of experiments of a control type, described in later paragraphs, have forced us to the paradoxic conviction that the genesis of the nerve lesions of vitamin A deficiency requires an essentially normal rate of growth of a normal nervous system and that mechanical injury, the result of a disproportion between the central nervous system and its bony enclosure, is the explanation.

That the nervous system in vitamin A deficiency continues to grow at approximately the normal rate is concluded from the following considerations: 1. The spinal cord grows 5 to 7 mm. too long relative to the spinal canal during the two weeks' period (42 to 56 days of age) in which the lesions develop. This represents slightly more than one week's normal growth for rats of this age. 2. If it is assumed that growth of bone is completely suspended during this two week period, the minimum growth rate of the spinal cord represented by the 5 to 7 mm. excess length in relation to the spinal canal would be about 60 per cent of the normal. If it is assumed that the growth of bone continues during this period at a normal rate, the 5 to 7 mm. excess length in relation to the spinal canal would represent a maximum growth rate of about 60 per cent greater than normal for the spinal cord. 3. Since histologic studies of the epiphyses of vertebrae and of other bones show severe retardation but nevertheless not complete suspension of endochondral bone growth in these vitamin A–deficient rats at 56 days of age, it is probable that the growth rate of the spinal cord is close to the mean of the aforementioned percentages.

Therefore, in the absence of any evidence obtainable by histologic and cytologic studies that this deficiency leads to accelerated nerve growth, we believe that the nervous system continues to grow at approximately the normal rate during this critical period. This is in distinct contrast to the relations preserved between the central nervous system and the skeletal system in rats whose growth has been stunted by other means.

Rats whose growth has been stunted because the rations of a complete diet were inadequate or because of other deficiencies (riboflavin and pyridoxine [vitamin B_6]) do not have nerve lesions and when dissected are found to have normal relations in size between the nervous system and the bony investment of this system. Rats severely stunted because their rations were inadequate and because they were deficient in riboflavin have invariably shown a spinal cord terminating at the upper level of the fifth lumbar vertebra or slightly above. Dissections of vitamin A–deficient rats which had shown paralysis of any degree

invariably revealed herniations of nerve roots, and if the paralysis had been marked or prolonged, herniations of the brain as well.

Histologically, the nervous systems of the paralyzed rats have always been normal, except for lesions accounted for by direct pressure. Attempts at regeneration by the axons involved in the mechanical injuries take place promptly and proceed for a time as in normal animals.

Age is important only as an index of the degree of development of the animal. We have kept rats at a low weight (50 Gm.) by means of inadequate rations of a complete diet until 14 weeks of age and then caused paralysis to develop by supplying large amounts of a diet deficient in vitamin A. Such rats started on the deficient diet acquired nerve lesions in the same period of time as did weanlings.

Rats on inadequate amounts of a diet deficient in vitamin A will not show paralysis if the general growth rate is sufficiently retarded. The inanition of continued vitamin A deficiency is manifested by cessation of growth and loss of weight at about 63 to 70 days of age. This is probably the explanation of the fact that the deformities of the nervous system reach their maximum in about ten weeks, for thereafter growth of the nervous system becomes retarded through inanition.

If at 42 days of age the vitamin A–deficient rats are placed on a diet containing carotene and the caloric intake is reduced so that their weight graphs parallel those of litter mate controls retained on the vitamin A–deficient diet, signs of nerve lesions do not appear. Many experiments of this sort were done. The rats were usually put to death at 63 days of age. Those retained on the vitamin A–deficient diet invariably became paralyzed and when dissected showed the characteristic relative overgrowth of the nervous system. The litter mates which received vitamin A with restriction of the quantity of food did not show signs of nerve lesions and on dissection the relations of nervous system to bone were within normal limits.

The retardation of bone growth in vitamin A deficiency has long been known.[34] Briefly, the cessation of bone growth is strikingly evident in the endochondral bone formation. To the present time it has not been shown that the character of the effect of vitamin A deficiency on bone growth is different from that due to general inanition. Considerable study by one of us (S. B. W.[35]) has not revealed in vitamin A deficiency any specific effect in the sequence or in the morphologic characteristics of the cells concerned in endochondral bone formation. Our present studies bring to light an unexpected promptness of effect on bone growth, which suggests that specific factors are involved, inasmuch as

34. Hess, A. F., and Pappenheimer, A. M.: J. Biol. Chem. **47**:395, 1921. Wolbach and Howe.[18]

35. Unpublished observations.

retardation of bone growth occurs before other consequences of general inanition are apparent.

The bones from the litter mate rats used for the study of the effects of carotene with restricted diet at 42 days of age have been utilized for microscopic studies after the rats were killed at 63 days of age. Owing presumably to the restriction of growth caused by reduction in the amounts of food, the effects of the administration of carotene at 42 days of age were not so pronounced as was expected. However, with rare exceptions, endochondral bone formation studied at the epiphyses of the lumbar vertebrae and at the lower end of the femur and the upper end of the tibia was somewhat more active in the rats that received carotene at 42 days of age and probably was the important factor in preventing the crowding of the central nervous system.

Since, however, the increased activity was not sufficient to produce a significant increase in the length of the spinal column by the end of the experiment as compared with the litter mate controls retained on the vitamin A–deficient diet, we could regard the prevention of paralysis in the rats of these experiments as possibly the result in part of the retardation of growth of the central nervous system consequent on the reduction in the quantity of food. We must remain indefinite on this point because we failed to make roentgenographic records of growth at intervals during the experiments.

Another indication of a possible specific effect of vitamin A on bone growth is the bony overgrowth in certain regions in the cranial cavity. Mellanby [4] was the first to describe the periosteal deposit of bone in relation to the capsules of the labyrinths of young dogs and elsewhere in the skull. We have confirmed Mellanby's observation and have found similar bony overgrowth in the guinea pigs chiefly if not wholly limited to bone in relation to the bony capsule of the labyrinth, as is the case with rats. We have been unable thus far to find similar bone changes in other parts of the skeleton in rats. We have found in our vitamin A–deficient paralyzed rats, in the inner table of the skull, immediately adjacent to sutures (fig. 5), bone formation in excess of that found in normal rats of the same age but conforming in general to the normal pattern.

Naturally, we have given careful study to the cortical bone which forms the spinal canal. In the vitamin A–deficient rats this layer is usually a trifle thinner than it is in normal and undernourished control rats. There is no suggestion of new bone formation like that in the skull adjacent to the periotic bony capsule. However, the evidences of growth sequences on the ventral side of the canal which reveal the mechanism by which the caliber of the canal enlarges are nearly or completely absent. It is probable that a retardation of the rate of increase of the caliber of the canal contributes to the crowding effect on the cord.

The work of Moore and associates [36] indicates that in calves there may be a different distribution of productive bone change.

For the present the bone proliferation of periosteal origin in the skull presents an enigmatic contrast to the other responses of bone in vitamin A deficiency with but a single suggestive premise: the fact that the bony capsule of the labyrinth attains adult size before birth.

The deduction we are forced to make is that in vitamin A deficiency in the young growing animal during the age periods of our experiments the central nervous system grows at a rate approximately normal for the species while bone growth is promptly retarded. Because of the weight increase of the vitamin A–deficient rats preceding and accompanying the early period of paralysis, it is possible that there occurs a disproportionate growth of all soft tissues in relation to the skeleton. Critical studies to evaluate this possibility have not been made. We find no evidence that an adequate amount of vitamin A or a deficiency of vitamin A affects the nervous system.

The only fact in regard to the epithelial responses in vitamin A deficiency pertinent to this study is that the characteristic change of keratinizing metaplasia in many locations is present by the time signs of paralysis appear, and may be pronounced at 56 days of age.

The keratinizing metaplasia of epitheliums is invariably produced in vitamin A–deficient rats regardless of age or degree of growth.

SUMMARY

The epithelial changes due to vitamin A deficiency are wholly unrelated to the presence or the absence of lesions of the nervous system.

The nervous lesions of vitamin A deficiency are wholly of mechanical origin, the genesis of which is a disproportionate growth of the central nervous system in relation to the bone which surrounds it.

Two conspicuous consequences of the disproportionate growth are noted: 1. There is overcrowding of the cranial cavity, resulting in distortion of the brain, dislocation toward the foramen magnum with herniation of the cerebellum therein, and multiple herniations of the cerebrum and cerebellum into the venous sinuses of the dura at sites of arachnoidal drainage structures. 2. There is overcrowding of the spinal canal with distortion of the spinal cord and herniations of nerve roots into intervertebral foramens and into bodies of vertebrae.

Rats whose growth is stunted because their rations of a complete diet have been inadequate or because of certain vitamin deficiencies (riboflavin and pyridoxine [vitamin B_6]) show a normal relation between the central nervous system and its bony investment.

36. Moore,[14] Moore and Sykes.[15]

In vitamin A deficiency in young animals the normal growth rate of the central nervous system is maintained until the effects of general inanition as shown by a decided retardation of gain in weight of the animal as a whole become apparent. Also, the regenerative power of the axons and presumably their physiologic potentialities are not impaired.

The early retardation of growth of bone is suggestive of a specific effect which, because of histologic observations, is probably chiefly operative in cartilage of the epiphyses. The vis a tergo of osteogenesis per se is not lost and possibly not impaired before the period of general inanition is reached, as suggested by the findings in pitted vertebrae and in regions adjacent to the periotic bony capsule.

Some observations on the arachnoidal villi of the rat are included.

NEUROPATHOLOGIC CHANGES IN EXPERIMENTAL CARBON DISULFIDE POISONING IN CATS

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Carbon disulfide is extensively used as a solvent in various industries, particularly in the making of artificial silk by the viscose process. In this industry, according to statistics of the Textile Economics Bureau 50,000 workers are employed in the United States, handling nearly 78,000,000 pounds (39,000,000 Kg.) of carbon disulfide yearly; with this extensive use of the solvent, the number of cases of carbon disulfide poisoning has consequently increased.

Emphasis has been repeatedly laid on the fact that in both acute and chronic intoxication the symptoms and signs of involvement of the nervous system are outstanding. In acute poisoning, among the immediate effects are mental changes with acute delirium. In chronic poisoning, according to Ranelletti's [1] work covering 100 patients, 80 per cent of the patients suffer from involvement of the nervous system; of these 52 per cent show mental changes, 10 per cent peripheral neural lesions and 7 per cent extrapyramidal signs. Peculiar tremors similar in character to those occurring in chronic epidemic encephalitis are frequent (Audo-Gianotti [2]; Negro [3]; Chiri [4]; Quarelli [5]). This last author found tremors in one third of 100 cases personally observed.

Although the importance of the involvement of the nervous system in this type of intoxication is widely recognized, descriptions of neuropathologic changes in human material are scanty and of little significance. Quensel [6] observed in a man 23 years of age who had shown an acute

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1. Ranelletti, A.: Arch. f. Gewerbepath. u. Gewerbehyg. **2**:664, 1932.
2. Audo-Gianotti, G. B.: Riforma med. **14**:1275, 1929; Presse méd. **40**:1289, 1932.
3. Negro, F.: Rev. neurol. **37**:518, 1930.
4. Chiri, C.: Med. d. lavoro **21**:4, 1930.
5. Quarelli, G.: Rass. d. previd. soc. **21**:10, 1934; Paris méd. **1**:533, 1937.
6. Quensel, F.: Monatschr. f. Psychiat. u. Neurol. **16**:48, 1904.

psychosis following exposure to a high concentration of carbon disulfide that the neuron cells were diffusely involved, showing varying degrees of advanced degeneration. He also observed small perivascular areas of softening in the cerebral cortex. Abe [7] in a case of chronic intoxication found lesions of cortical neuron cells with varying glia reaction, as well as widespread areas of necrosis in which demyelination, accumulation of gitter cells, endothelial alteration of the walls of blood vessels, and perivascular infiltration were in progress. The cerebellar cortex was also involved, with loss of Purkinje cells and increase in Bergmann's cells. In a man who had shown symptoms of intoxication for over a year, Fujii and Hirose [8] noted diffuse degeneration of the nerve cells of various degrees associated with proliferation of the glia. Alpers [9] reported 2 cases of carbon disulfide poisoning. In the first case there were marked sclerosis of the cerebral arterioles and patchy loss of ganglion cells, particularly in the frontal areas, cell shrinkage of the globus pallidus and putamen, and evidence of peripheral neuritis. In the second case there was relatively little except neuritis, the cortical changes being minimal.

The neuropathologic findings following experimental intoxication in laboratory animals have been more extensive. Since Delpech's [10] first experiments in 1863, numerous investigators have contributed to this problem. Köster,[11] working with rabbits and using varying doses of poison, found destruction of myelin in the peripheral nerves and degenerative changes in the nerve cells of the cerebral cortex and cerebellum. Koelsch [12] in a study of cats described diffuse degenerative changes of the ganglion cells and the peripheral nerves following exposure in an atmosphere containing 10 to 11 mg. of the toxic gas per liter. Ranelletti [1] and Audo-Gianotti [13] also reported similar degenerative changes of the nerve cells, the latter stressing the involvement of the neurons of the basal ganglions. Wiley and co-workers [14] found cerebral lesions characterized by marked edema, perivascular hemorrhages, degeneration of the ganglion cells and small cystic formations. Recently Baumann [15] found no changes in the brain of a cat which was

7. Abe, M.: Jap. J. M. Sc. Tr., VIII, Int. Med., Pediat. & Psychiat. **31**:1, 1933.

8. Fujii, O., and Hirose, T.: Mitt. d. med. Gesellsch. zu Tokio **48**:1492, 1934.

9. Alpers, B. J.: Arch. Neurol. & Psychiat. **42**:1173, 1939.

10. Delpech, M. A.: Ann. d'hyg. **19**:65, 1863.

11. Köster, G.: Arch. f. Psychiat. **32**:569, 1899.

12. Koelsch, F.: Jahresk. f. ärztl. Fortbild. (no. 9) **22**:29, 1931.

13. Audo-Gianotti, G. B.: Rassegna di med. appl. lavoro indust. **2**:281, 1931.

14. Wiley, F. H.; Hueper, W. C., and von Oettingen, W. F.: J. Indust. Hyg. & Toxicol. **18**:733, 1936.

15. Baumann, C.: Ztschr. f. d. ges. Neurol. u. Psychiat. **166**:568, 1939.

exposed for ten hours to the poison, while in a second cat, chronically intoxicated, there were aspecific degenerative changes of the neuron cells.

Alpers and Lewy [16] produced carbon disulfide intoxication in 9 dogs. Most prominent among the neurologic signs were tremor and ataxia. In all animals there were cellular changes extending to the cerebral cortex, corpus striatum and Purkinje cells. The cortical structure was remarkably well preserved in all cases. The glia showed only regressive changes, no proliferation being observed. Throughout the cortex there were also extensive vascular changes consisting of proliferation of the endothelium and thickening and hyalinization of the arteriole wall.

All these experiments undoubtedly bear out the affinity of carbon disulfide for the nervous system; however, no lesion has been recorded in the literature which might be considered in itself characteristic of this type of poisoning. Further investigation therefore appeared justified.

Summary of Experimental Data

Cat	Total Hours of Exposure	Total Days	Hours per Day
1	19	24	2-3
2	38	30	2-3
3	40	27	2-5
4	74	51	1-3
5	65	92	½-1

EXPERIMENTAL PROCEDURE

Cats were used. Each animal was placed for varying periods in a large box of about 750 liters capacity, with a glass front to permit observation. Between 6 and 7 Gm. of liquid carbon disulfide was placed in the box, in a large dish over a slightly warmed electric stove in order to facilitate rapid evaporation. After complete evaporation 8 to 10 mg. of carbon disulfide per liter was present in the box. The volume of the box guaranteed a sufficient supply of oxygen so that the results could not be attributed to anoxia. The table shows the number of each cat and the duration of exposure.

The clinical signs shown by the cats during exposure to the gas varied. Increased salivation and dyspnea were frequent. Vomiting was observed only occasionally. Restlessness and excitement were common in the first stages of intoxication; at more advanced stages apathy was observed, and in a few instances, coma. Tremors and muscular jerks were frequent, but convulsions were observed only in a single instance. All these symptoms disappeared in a few hours after the animals were taken out of the box. During the interval, apart from a certain apathy the most striking symptom was a fine tremor involving the head and at times the whole body. This tremor appeared after several days of exposure and was almost continuous. It was especially marked in 3 cats (1, 4 and 5).

Each animal was killed with ether and an immediate autopsy made.

16. Alpers, B. J., and Lewy, F. H.: J. A. M. A. **115**:59, 1940.

HISTOLOGIC OBSERVATIONS

A brief description of the main pathologic alterations in the brain of each animal follows:

CAT 1.—In the Nissl preparation the normal structure of the cerebral cortex was everywhere well preserved, though one could easily recognize proliferation of glia elements in the lamina molecularis. This involvement of the lamina molecularis, which was common to all the cases, is well illustrated by figure 1, taken from cat 5. In the cortex one found in addition a definite increase in the number of small blood vessels, whose walls were thicker than normal. The pia was generally thickened and occasionally adherent to the subjacent cortex. High power showed varying degrees of injury of single neuron cells, consisting mainly of swelling of the cellular bodies and chromatolysis. In a few instances, colliquation of the neuron cells could be observed. Shadows and remnants of nerve cells were often encountered. This involvement of nerve cells was diffuse all over the cortex, though the inner and the outer layer may be considered as slightly more damaged.

The changes seemed to be more pronounced in certain cortical areas than in others. For instance, in the temporal lobe, the cornu ammonis and the occipital lobe the lesions of the nerve cells were more evident. In the substantia nigra there was a definite increase in the number of glia nuclei, as well as rarefaction of nerve cells. In the corpora quadrigemina one found a combination of nerve cell destruction, neuronophagia, glia proliferation and productive endarteritis. This productive endarteritis was characterized by an increase in the number of small blood vessels and a thickening of their walls. At times the outer layers were thickened; at others it was the intima which appeared stratified. Such characteristic vascular reaction was found scattered all over the brain tissue, predominating at times in the gray matter, at others in the white.

In the white matter it was especially in the anterior portion of the internal capsule and in the cornu ammonis that one found most of the increase in number of glia nuclei and thickening of blood vessels.

The myelin sheaths in the cortex and underlying white matter showed no significant changes. No fat was present in the Herxheimer preparation. Hortega stains showed acute swelling of the oligodendroglia but no appreciable changes in the microglia.

The basal ganglions and the thalamic nuclei showed only occasional swelling and chromatolysis of single neuron cells but definite thickening of blood vessels.

As to the cerebellum, the Spielmeyer preparations disclosed a large patch of demyelination involving the cerebellar nuclei bilaterally (fig. 2). In a Herxheimer preparation this patch of demyelinization was found to be filled with large amounts of fatty substances, mainly collected within the cytoplasm of gitter cells. This large focus of partial softening included the dentate nucleus and the roof nuclei. The neuroglia cells appeared increased in size and number, and some degenerative forms were observed. The blood vessels within the lesion showed thickened walls, with endothelial cells swollen and often proliferating. There were no hemorrhages. No thrombi were observed. The axis-cylinders in the center of the lesion had disappeared.

Involvement of the Purkinje cells, consisting mainly of homogenization of the cytoplasm, neuronophagia and disintegration of some of the cellular elements, was encountered in diffuse distribution. In addition, one noted here and there clumping of cells in the granular layer, i. e., conglutination, as described in other exogenous toxic conditions (fig. 3, taken from cat 4).

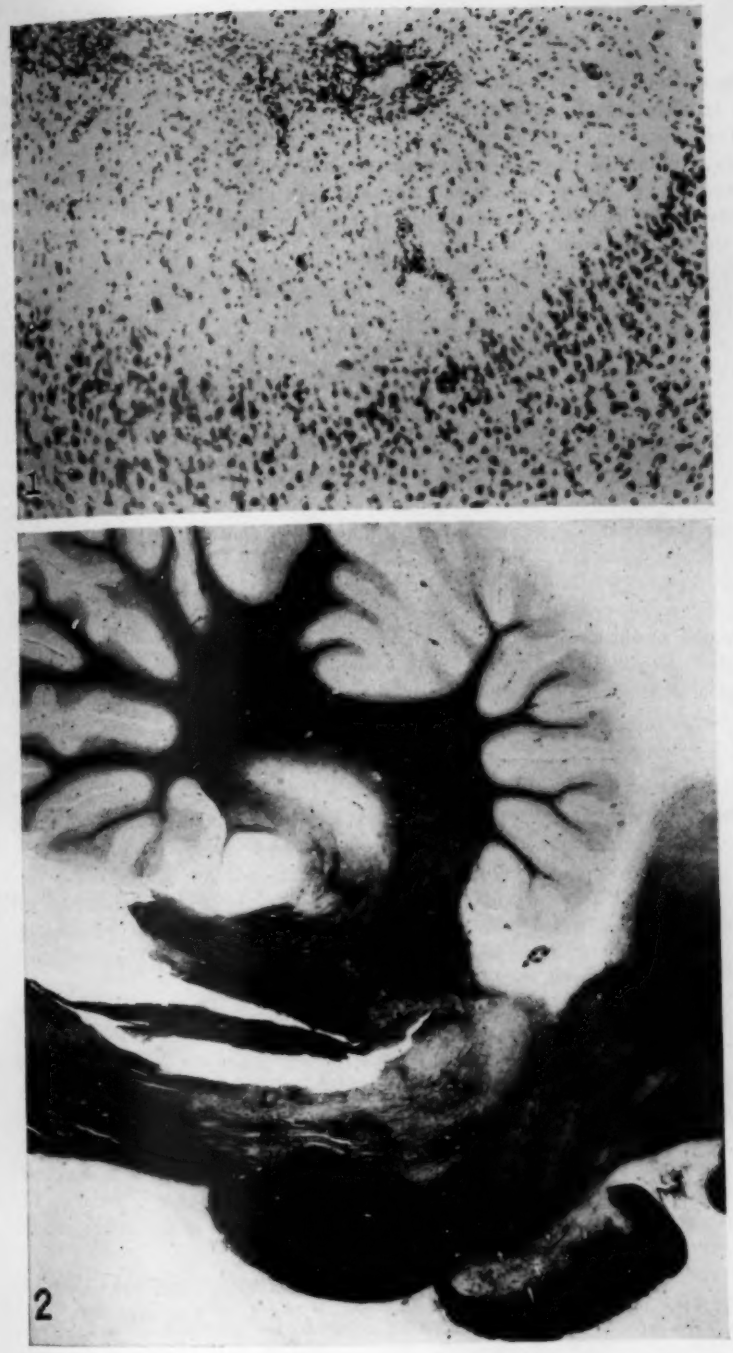

Fig. 1.—Proliferation of blood vessels and glia cells in the lamina molecularis. Nissl stain.

Fig. 2.—Secondary demyelination following softening of the cerebellar nuclei, taken from cat 5. Spielmeyer method for myelin sheaths.

The vestibular region was also the seat of interesting changes, consisting of nerve cell degeneration, glia reaction and thickening of blood vessels. It seemed that Deiters' nucleus and the nucleus triangularis were the most severely involved.

Glia proliferation was also noticed surrounding the superior cerebellar peduncle, in the group of nerve cells bordering this structure. The superior cerebellar peduncle itself disclosed secondary degenerative changes.

A slight involvement of the superior olive was noticed.

CAT 2.—Fundamentally, the same changes were found as in cat 1. The intensity of the nerve cell involvement was, however, less pronounced than in cat 1. The topographic distribution of the changes did not vary substantially from that in the previous animal. One may add that in cat 2, the olfactory bulb disclosed changes involving some of the mitral cells and cells of the olfactory glomeruli.

In the cerebral cortex the same glia reaction and vascular proliferation as described in cat 1 were noticed in the lamina molecularis. In the sigmoid gyrus some of the large pyramidal motor cells had undergone complete degeneration, resulting in rarefaction of such cells. Others disclosed chromatolysis and pyknosis.

In the corpora quadrigemina an increase of glia nuclei, thickening of the blood vessels and proliferation of the small ones constituted the main pathologic features.

In the cerebellum the region of the cerebellar nuclei disclosed typical softening and accompanying repair. Where the softening was not present, glia proliferation and vascular productive changes were noted (fig. 4).

In the cerebellar cortex one found homogenization, neuronophagia and disintegration of some of the Purkinje elements; there was only very slight clumping of granular cells. Degenerative changes, though less marked, were also present in the vestibular nuclei.

CAT 3.—Even though this animal had been exposed to the action of carbon disulfide for a total number of hours greater than that of the exposure of cat 1, the changes in the nervous system were decidedly less pronounced.

The meninges were less thickened, and the glia reaction and vascular proliferation were less pronounced. The nerve cell changes were less intense and consisted generally of diffuse chromatolysis, swelling of the cytoplasm and pyknosis. Some nerve cells were undergoing severe degenerative changes. Here and there were some edematous cells, particularly in the outer layers.

The vascular changes were also generally less pronounced in this animal with the exception of the corpora quadrigemina and the anterior nucleus of the thalamus.

In the cerebellum, no softening was found in the cerebellar nuclei. Here the process was limited to glia reaction and vascular thickening and proliferation. Occasional hyaline degeneration of small blood vessels was noted.

The same increase in number of glia nuclei, but with less proliferative vascular change, was observed in the vestibular nuclei, particularly the nucleus triangularis. Myelin sheath preparations of these regions disclosed some breaking down of myelin sheaths and some rarefaction. No gitter cells or free products of fatty degeneration were encountered.

In the cerebellar cortex the Purkinje cells were better preserved and the granular cells were quite intact.

The peripheral nerves showed no significant changes.

CAT 4.—Of all the animals in this series, this one disclosed the most pronounced changes of nerve cells, ranging from acute swelling to the severe type of degeneration described by Nissl (fig. 5). The scattered changes did not lead to any

appreciable disturbance in the structure of the cortex. One found, however, advanced disintegration of nerve cells, particularly in the surrounding of blood vessels, resulting in small acellular areas (fig. 6). Only here and there was shrinkage of nerve cells detected. All over the cortex, particularly in the lamina molecularis, one observed the usual vascular reaction as described in other cases, though more pronounced.

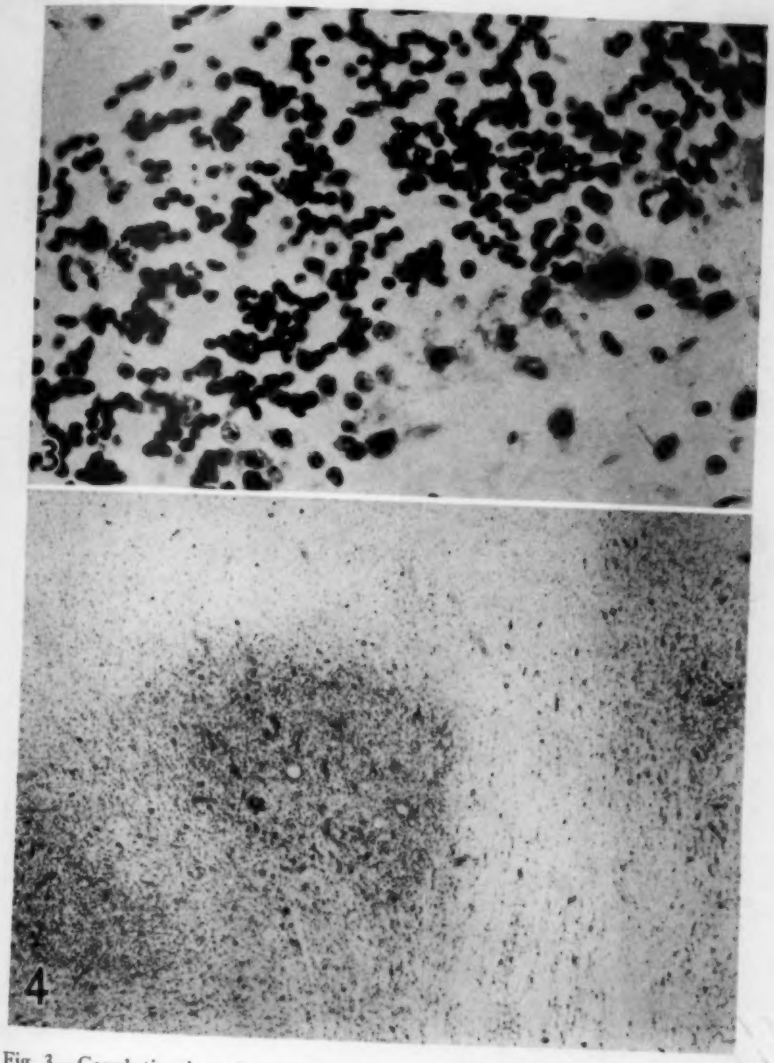

Fig. 3.—Conglutination of granular cells in the granular layer of the cerebellum and marked degeneration of Purkinje cells, taken from cat 4. Nissl stain.

Fig. 4.—Proliferation of glia nuclei and blood vessels and endarteritis in areas of the cerebellar nuclei where no definite softening is present. Notice in the picture marked involvement of Deiters' nucleus. Nissl stain.

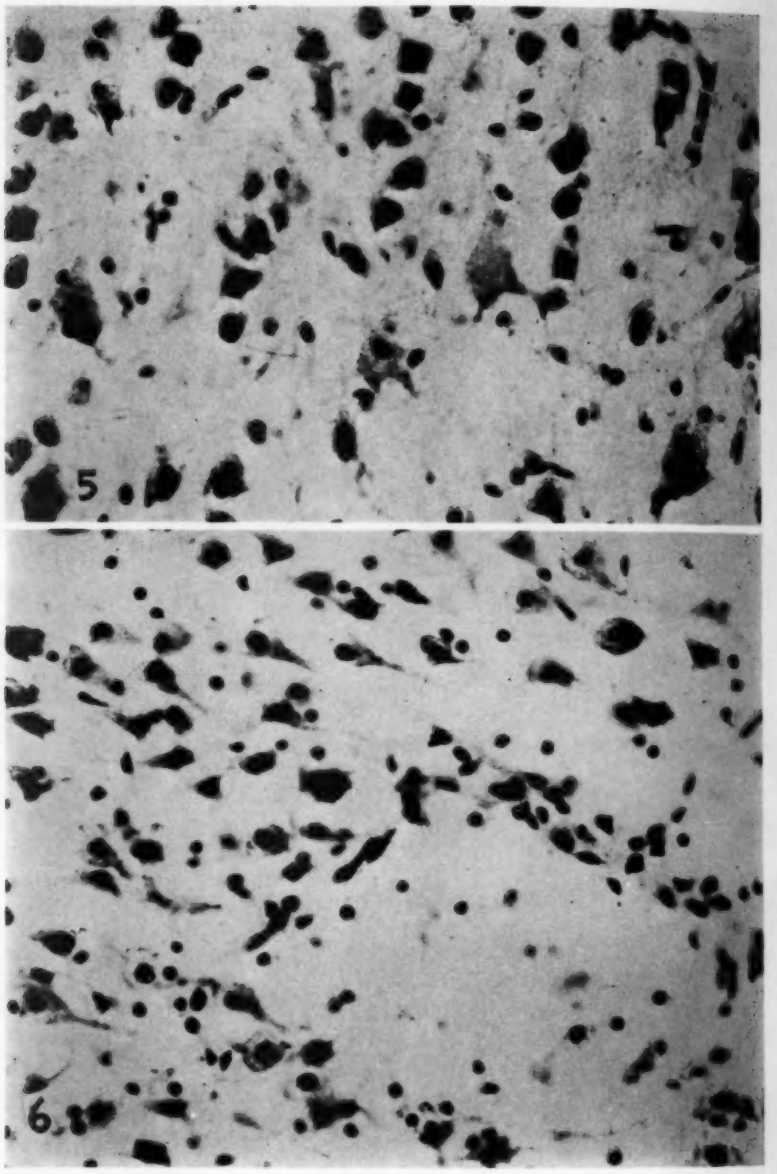

Fig. 5.—Severe type of Nissl's degeneration in nerve cells of the temporal cortex. Nissl stain.

Fig. 6.—Acellular areas resulting from disappearance of nerve cells. Note shadow cells remaining in the areas. Nissl stain.

The white matter, particularly that of the frontal lobes, internal capsule and temporal lobes, disclosed here and there considerable increase in number of glia nuclei and proliferation of small blood vessels. The corresponding gray matter of the immediate vicinity disclosed the most severe degenerative changes observed in nerve cells. In the cornu ammonis clusters of cells of the lamina pyramidalis presented acute degenerative changes.

The caudate nucleus, the thalamus, particularly its medial portion, and the corpora quadrigemina disclosed marked proliferation of blood vessels, thickening of blood vessels and occasional hyaline degeneration. In the mamillary body the nerve cells were mostly swollen, with enlarged swollen nuclei.

In the lenticular nucleus there was a typical large area of softening involving the globus pallidus from the anterior third to the posterior end (fig. 7). The cephalic third of the putamen was apparently normal, the softening appearing first in the medial portion.

In the substantia nigra there was a small area of softening located in the medial portion of the substantia nigra, invading both the dorsomedial group of cells and the subjacent pes pedunculi. In the corpus geniculatum laterale a few severely degenerated nerve cells were encountered. In the red nucleus, which was generally well preserved in all the cases, only a few large cells of the pars magnocellularis disclosed slight degenerative changes.

The cerebellum showed an area of softening similar to that found in cats 1 and 2. It involved, on both sides, the region of the dentate as well as of the other adjacent cerebellar nuclei. The vestibular nuclei in this case also disclosed nerve cell degeneration, increase in number of glia nuclei and vascular proliferation.

Cat 5.—In the cortex, thickening of the pia with proliferation of glia nuclei and of blood vessels in the lamina molecularis was a common feature.

A feature encountered more frequently in this case was the presence of numerous areas of cellular rarefaction surrounding blood vessels in the outer and inner layers of the cortex. Areas of rarefaction were particularly evident in the frontoparietal cortex. In the sigmoid gyrus, rarefaction of large motor pyramidal cells was noticed.

In the caudate nucleus, increase in number of glia nuclei plus vascular proliferation and thickening of blood vessels was very definite (fig. 8). No appreciable change was present in the lenticular nucleus, whereas in the claustrum the blood vessels were thickened and slightly proliferated.

In the corpora quadrigemina, particularly in the anterior one (fig. 9), and in the corpus geniculatum mediale, vascular hypertrophy and hyperplasia, as well as nerve cell degeneration, were encountered.

In the white substance there were marked increase in number of glia nuclei and increase in number and thickening of blood vessels, particularly in the anterior branch of the internal capsule, the parietal area and the cornu ammonis.

In the cerebellum a typical area of softening was seen to involve the cerebellar nuclei bilaterally. Hematogenous elements were present in the area of softening, which was defined and limited largely to the lateral cerebellar nuclei. However, in the adjacent intact white matter of the cerebellum a few blood vessels were surrounded by gitter cells and lymphocytes. Thrombosis was not observed.

In the cerebellar cortex the layer of granules was rather well preserved; the Purkinje cells, on the other hand, disclosed diffuse degenerative changes, mainly homogenization, leading here and there to disappearance of the cells.

In the superior cerebellar peduncle one could follow the typical secondary degeneration resulting from involvement of the dentate nucleus (fig. 10).

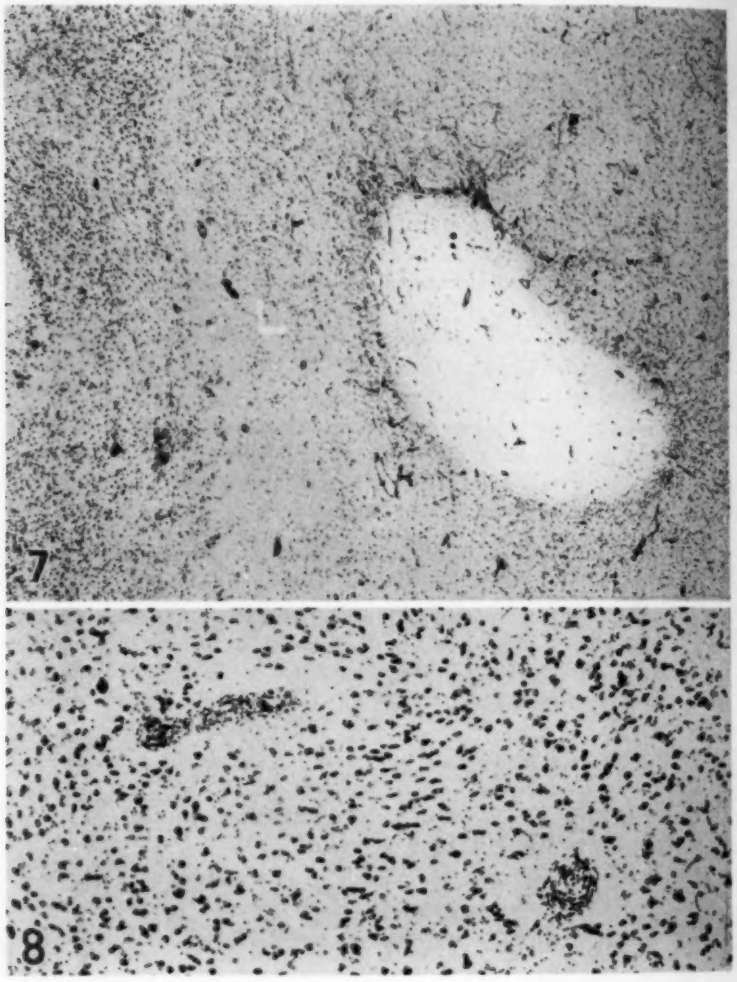

Fig. 7.—Area of softening in the lenticular nucleus involving both the putamen and part of the globus pallidus. Nissl stain.

Fig. 8.—Marked thickening of walls of blood vessels of the caudate nucleus. Nissl stain.

In Deiters' nucleus, vascular proliferation and increase in the number of glia nuclei were noted. In the remaining portion of the medulla some of the large cells of the formatio reticularis disclosed degenerative changes.

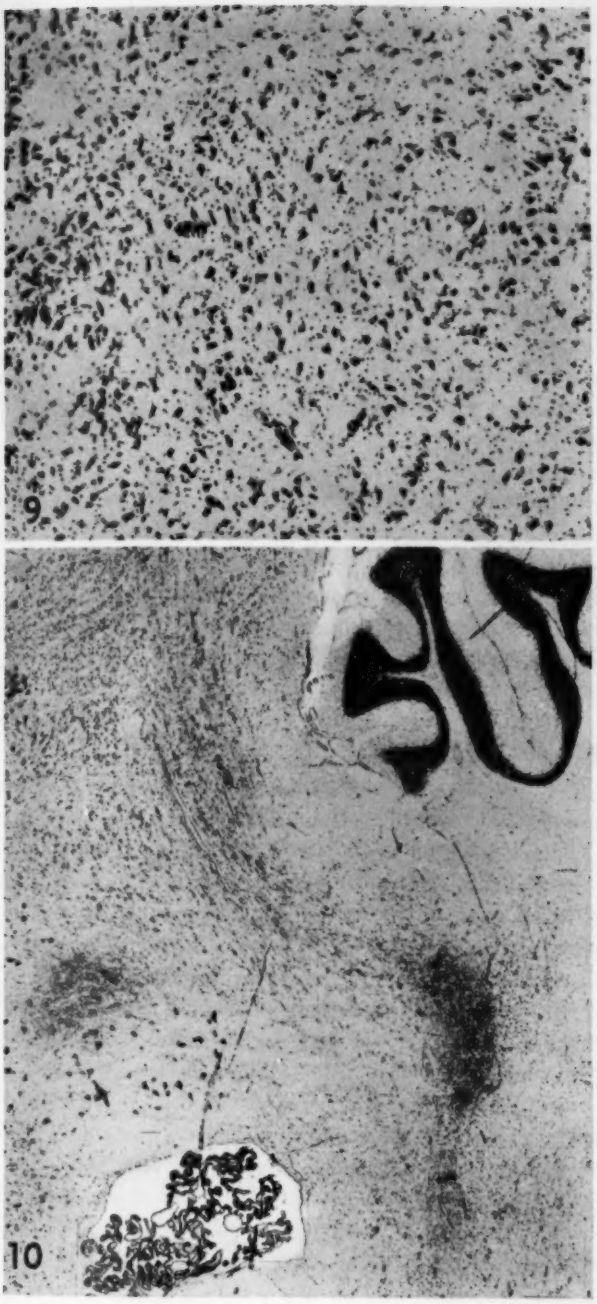

Fig. 9.—Proliferation of blood vessels and glia in the anterior quadrigeminal body. Nissl stain.

Fig. 10.—Degenerative involvement of the dentate nucleus and Deiters' nucleus. Note secondary degeneration in the superior cerebellar peduncle (sagittal section). Nissl stain.

COMMENT

From the changes in the 5 cats submitted to experimental inhalation of carbon disulfide it appears that the fundamental process in carbon disulfide poisoning is represented by (1) vascular involvement and (2) neurocellular involvement. The vascular involvement is represented by diffuse thickening of blood vessels and proliferation of capillaries. The thickening of blood vessels takes place at the expense of both the lining endothelium and the external vascular layers.

The vascular change is generalized, involving the blood vessels of most of the cortical areas, with some predilection for the outer molecular layer. In this layer one often sees the small blood vessels entering from the pia, unusually thick and with a definite increase in the number of their branches.

Of the subcortical structures, the caudate and the lenticular nucleus seem to disclose a more pronounced vascular change than other areas. Less pronounced is the involvement of the thalamus. In the mesencephalon, the red nucleus seems rather free from vascular involvement, whereas such an involvement is present in the substantia nigra and in the dorsal portion of the pes pedunculi. In the tectum of the mesencephalon and pons, the quadrigeminal bodies seem to constitute an area where the vascular proliferation is more marked than in any other area of the brain stem.

In the medulla, the area of the vestibular nuclei seems to be another area of predominance of the vascular lesion. It is particularly the triangular nucleus and Deiters' nucleus which are most frequently involved.

Finally, in the cerebellum, the nuclei dentatus, globosus, emboliformis and fastigii disclose severe vascular involvement.

This involvement of the blood vessels leads, here and there, to complete occlusion of the lumen, as a result of which areas of softening may result. Concerning the topographic distribution of such softenings, we must mention the fact that they are almost always localized to the cerebellar nuclei and that only in a single instance was an area of softening found in the lenticular nucleus and in the medial portion of the substantia nigra (cat 5). This happens to have been the animal that was exposed for the longest number of hours to the action of carbon disulfide, namely, seventy-four hours in the course of fifty-one days.

This question of the frequent occurrence (in 4 of the 5 cats) of softening in the cerebellar nuclei following exposure to carbon disulfide raises the interesting question of the genesis of the areas of softening as well as of the predominance of the pathologic process.

Concerning the question of genesis, we feel that vascular involvement is fundamentally responsible for the softening inasmuch as the

vascular involvement leads ultimately to more or less complete occlusion of the vessels and shutting off of the vascular supply. We have failed to find in the softening any microscopic character pointing to a different genesis. We found in the Spielmeyer preparation destruction of myelin sheaths generally limited to the area outlined by the neuron cells of the various cerebellar nuclei, the dentate being chiefly involved in some instances, the roof nuclei in others. The neuron cells of the various cerebellar nuclei show varying degrees of degenerative changes, ranging from chromatolysis to colliquation. In hematoxylin-eosin and Nissl preparations within the area of softening we found a large number of cellular elements, the majority representing compound granular corpuscles, which in the Herxheimer preparations appeared to be laden with fat. Occasionally, a few lymphocytes and plasma cells were also observed surrounding some of the blood vessels. In all the lesions, there were, in addition to the disintegration of nerve elements, reaction and proliferation on the part of the glia, microglia and mesenchymal elements, indicating a definite process of organization and repair. Thus one finds marked neoformation of capillaries and mesenchymal network, particularly intense in the early stages of the organization. In more advanced stages the mesenchymal reaction is substituted by considerable glia reaction, particularly of the fibrous type.

Concerning selectivity, we should not speak so much of it as of predominance of the lesions in the cerebellar nuclei. As mentioned before, one finds the vascular reaction distributed all over the cortical, diencephalic, mesencephalic and rhombencephalic structures. The severity of the process is definitely more pronounced in the cerebellar nuclei, where it almost invariably leads to softening. It is difficult, however, to draw a definite line between predominance and selectivity of a pathologic process, and therefore we shall consider the predominance of the lesions in the cerebellar nuclei in the same light as the selectivity.

To be sure, the fact that certain regions of the brain are selectively vulnerable to toxic or infectious agents is well substantiated by numerous findings in both human and experimental neuropathology; thus, the predominance of lesions of the substantia nigra in epidemic encephalitis, the particular susceptibility of the anterior horn in poliomyelitis and the softening of the globus pallidus in carbon monoxide intoxication are well known instances.

The cause of this selectivity or predominance is still obscure. Since differences in architectonic structures might be correlated with differences in physicochemical constitution, it is conceivable that unequal inclination to disease of the different structures of the brain will occur, resulting in predominant or selective action of certain morbid agents on certain structures (theory of pathoclisis of Vogt and Vogt[17]).

17. Vogt, O., and Vogt, C.: J. f. Psychol. u. Neurol. **28**:1, 1922.

Although of considerable theoretic importance and doubtless of use as a working hypothesis, this theory needs the support of more convincing evidence. For instance, no proof has yet been brought forward that the cortex of the brain differs physicochemically from the globus pallidus, leaving thus unsolved the problem as to why the latter is more susceptible than the former to various types of intoxication. As far as the cerebellar nuclei are concerned, data bearing on their physicochemcial characteristics are lacking. Any attempt to explain the lesion on the basis of the theory of pathoclisis is therefore purely speculative.

It will be noted, on the other hand, that the dentate nucleus, together with the cornu ammonis, the pallidal system and the inferior olivary body, is particularly vulnerable to disturbance of blood supply. It seems, therefore, reasonable to attribute importance to the "vascular factor" (Spielmeyer [18]) in the genesis of the dentate lesion due to carbon disulfide. That this vulnerability is dependent on inherent qualitative or quantitative structural peculiarities of the capillary network as demonstrated in the cornu ammonis is possible, but no such structural peculiarity in this region has been established as yet. We are inclined, however, to believe that in carbon disulfide poisoning the softening of the cerebellar nuclei is closely related to the vascular lesions, our contention being supported by the fact that most of the changes have a perivascular distribution. If to the structural changes of the vascular system in the cerebellar nuclei additional functional vascular changes are added in the direction of functional vasospasms, it is difficult to establish.

One might mention that as far as the dentate nucleus is concerned, that region is particular rich in iron (Guizzetti [19]; Spatz [20]). Since iron plays an important role as oxygen activator in the respiratory enzymatic system of the cells, it is possible that the metabolism of the dentate nucleus might be particularly active. Consequently this region would be more sensitive than other areas to interferences with oxygen supply brought about by vascular impairment. The area of softening in the dentate nucleus could then be explained on the basis of the structural involvement of the vascular system, which in turn causes particularly severe parenchymatous alterations because of the particular chemical constitution of this region. Incidentally, a hypothesis of this type would take into consideration both Vogt's theory of pathoclisis and Spielmeyer's theory of a vascular factor. Whether such speculative arguments as brought forward in connection with the dentate

18. Spielmeyer, W.: Ztschr. f. d. ges. Neurol. u. Psychiat. **118**:1, 1928.
19. Guizzetti, P.: Riv. di pat. nerv. **20**:5, 1915.
20. Spatz, H.: Ztschr. f. d. ges. Neurol. u. Psychiat. **77**:261, 1922.

nucleus also apply to the other cerebellar nuclei and to the vestibular nuclei (particularly Deiters'), which are also a seat of predilection of the changes, we are not prepared to state, not being aware of any related conclusions based on the angioarchitectonics or biochemical structure of these areas.

Concerning the relationship between the time of exposure to the gas and the occurrence of softening in the cerebellum, we must mention the fact that in cat 3, exposed for forty hours to the action of gas, no softening was observed in the cerebellar nuclei, constrasting with the occurrence of such softening in cats 2 and 1 exposed, respectively, for thirty-eight and nineteen hours. It follows necessarily that other factors, in addition to the intensity of the action of the exogenous factor, must play a role in the determination of the severity of the lesion.

The next important question in regard to the lesions of carbon disulfide poisoning in the central nervous system is the occurrence of diffuse lesions of nerve cells all over the brain. The involvement of the nerve cells is observed all over the cortex and in the subcortical structures in the cerebrum and cerebellum, as well as in the mesencephalon, medulla and pons. The involvement consists mostly of degenerative changes of the neuron cells, ranging from chromatolysis and acute swelling to severe degeneration of the type described by Nissl. Some of the cells appear vacuolated, others disclose pyknosis in the midst of a poorly stained cytoplasm, and a few others disclose shrinkage of the cell body and of the nucleus, both structures being rather deeply stained. In the most severe cases, rarefaction of cells, acellular areas, shadows of cells and remnants of nerve cells are found diffusely distributed.

A certain number of cells disclose hydropic changes with swelling of the cell body and nucleus and formation of a clear halo between the nucleus and the cytoplasm. Some of these cells are particularly found in the outer layer of the cortex in correspondence to the zone of entry of the pial blood vessels, which disclose characteristic productive changes. In most of the areas, such as the caudate nucleus, corpora quadrigemina, vestibular nuclei and cornu ammonis, where the vascular changes are more pronounced, the nerve cell changes are also more distinct. Generally speaking, the chromatolysis with pallor of the cytoplasm and slight pyknosis is one of the most common neurocellular findings.

Such degenerative changes of the neuron cells as are reported here have been repeatedly described and commented on by the majority of investigators of experimental intoxication with carbon disulfide. They need, therefore, no special emphasis. The mechanism by which these morphologic changes of the neurons are brought about is likely to be found, among other factors, in the well known narcotic action of carbon

disulfide, similar in nature to the action of ether and chloroform. Evidence has been brought forward supporting the view that these substances act directly on the neuron cell through interference with cellular respiratory enzymatic systems. It is then conceivable that repeated and prolonged histotoxic anoxia will bring about morphologic changes demonstrable in the Nissl preparation.

Another mechanism of the genesis of the neurocellular changes resides undoubtedly in the vascular involvement with its related disturbance in circulation due to the narrowing and occasional occlusion of small vessels. One need not emphasize again the details of such an occurrence which is common to so many pathologic conditions.

In correspondence to the area where the vascular involvement is more pronounced and where consequently the nerve cell changes are more accentuated, a definite increase in number of glia nuclei is found, particularly so in the white matter, where clumps or longitudinal rows of compact nuclei presumably of oligodendroglial cells are found along the course of the blood vessels.

SUMMARY

Five cats were exposed to varying doses of carbon disulfide. The most important changes in carbon disulfide poisoning consist in (*a*) diffuse vascular involvement of the productive type, i. e., proliferation of capillaries and hypertrophy of the walls of blood vessels often leading to endarteritis, and (*b*) diffuse neurocellular changes ranging from chromatolysis to severe degeneration, diffusely scattered all over the brain and cerebellum.

Both cortical and subcortical structures are involved. The lesions are particularly evident in the corpora quadrigemina, the cerebellar nuclei and the vestibular nuclei. The region of the cerebellar nuclei constitutes an area where the change is definitely predominant and in 4 of 5 cases the pathologic involvement leads to bilateral softening of this region. Next to the cerebellar nuclei, the vestibular area is the one most intensely involved. In an animal in which the exposure to the action of the gas was most prolonged, softening was also found in the lenticular nucleus and in the substantia nigra.

The significance of these findings is briefly discussed with reference to their genesis and to the problem of regional vulnerability of the central nervous system.

GROWTH PROCESSES IN MAMMARY GLANDS OF MICE OF STRAINS DIFFERING IN INCIDENCE OF MAMMARY CARCINOMA

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Former investigations have shown that in the development of mammary gland carcinoma in mice essentially two kinds of factors cooperate: (1) hereditary factors and (2) ovarian hormones, which act as stimulating factors. A quantitative relation exists between these two influences. In a general way it may be stated that the stronger the hereditary tendency the less the stimulation required in order to produce cancer, and there is also an inverse ratio between the intensity of stimulation needed and the strength of the hereditary tendency: S (stimulation) × H (hereditary tendency) = C (cancer). There are strong indications that both stimulating and hereditary factors are effective because they regulate and may increase the growth activity of the tissues. If a certain intensity of growth has been exceeded, the normal tissue equilibrium is changed into the cancerous equilibrium. As a possible explanation of this transformation, it was suggested that the latter depends on the new formation or increased production, within the affected tissue, of a growth substance which is propagated autocatalytically.[1]

It seemed possible, then, that the difference in the incidence of mammary gland cancer in different strains of mice is contingent, at least in part, on a differential responsiveness of the mammary gland

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1. Lathrop, A. E. C., and Loeb, L.: Proc. Soc. Exper. Biol. & Med. **11**: 34, 1913; J. Exper. Med. **22**:646, 1915; J. Cancer Research **1**:1, 1916. Loeb, L.: Science **43**:293, 1916; J. M. Research **40**:477, 1919; Am. J. M. Sc. **159**:781, 1920; Am. Naturalist **55**:510, 1921; J. Cancer Research **8**:274, 1924; Heredity and Internal Secretion in the Etiology of Cancer, in Report of the International Conference on Cancer, London, 17th-20th July 1928, New York, William Wood & Company, 1928, p. 48.

tissue in these strains to hormonal stimulation of growth. However, other factors had also to be considered. It was conceivable that the strains with high and low mammary gland cancer rates differed in the rapidity with which estrogenic hormones are eliminated. But the fact that the incidence of carcinoma of the vagina and cervix, a tumor which seems also to depend on the action of estrogenic hormones, does not correspond to the incidence of mammary gland carcinoma in various strains of mice makes this interpretation improbable.[2] It was furthermore possible that there were differences in the sexual cycle in various strains and that in mice with a high cancer incidence the estrogenic hormones had a chance normally to act over a longer period than in others. However, the comparative study of the sexual cycle in various strains of mice did not indicate such differences.[3]

In order to elucidate further the relation between differences in the responsiveness of the mammary gland tissue to growth stimuli and the origin of mammary gland carcinoma in mice, we began, about eight years ago, a comparative study of the growth reactions of the mammary gland in various strains. For this purpose, we made a microscopic examination of the mammary glands and of other organs, in particular the endocrine glands, of mice of different ages and in different strains. We also examined the corresponding organs of normal male and female, as well as of ovariectomized, mice which had received subcutaneous injections of estrogen over various periods. We have communicated some of the conclusions arrived at in several previous publications.[4] Gardner, Diddle, Smith, Allen and Strong,[5] as well as Bonser,[6] likewise studied the differences in the growth of the mammary gland in different strains, the former, by dissecting the mammary gland system of control mice and also of mice given injections of

2. Moskop, M.; Burns, E. L.; Suntzeff, V., and Loeb, L.: Proc. Soc. Exper. Biol. & Med. **33**:197, 1935; Am. J. Cancer **26**:761, 1936.

3. Loeb, L., and Genther, I. T.: Proc. Soc. Exper. Biol. & Med. **25**:809, 1928. Bonser, G. M.: J. Path. & Bact. **41**:33, 1935. Burns, E. L.; Moskop, M.; Suntzeff, V., and Loeb, L.: Am. J. Cancer **26**:56, 1936; **30**:47, 1937.

4. Loeb, L.; Burns, E. L.; Suntzeff, V., and Moskop, M.: Canad. M. A. J. **35**:117, 1936; Am. J. Cancer **30**:47, 1937. Loeb, L.: Acta Union internat. contre cancer **2**:148, 1937.

5. Gardner, W. U.; Diddle, A. W.; Allen, E., and Strong, L. C.: Anat. Rec. **60**:457, 1934. Gardner, W. U.; Smith, G. M., and Strong, L. C.: Proc. Soc. Exper. Biol. & Med. **33**:148, 1935. Gardner, W. U.; Smith, G. M.; Allen, E., and Strong, L. C.: Arch. Path. **21**:265, 1936. Gardner, W. U.: Some Fundamental Aspects of the Cancer Problem: Symposium, New York, Science Press, 1937, p. 67. Gardner, W. U.; Strong, L. C., and Smith, G. M.: Am. J. Cancer **37**: 510, 1939.

6. Bonser, G. M.: J. Path. & Bact. **42**:169, 1936.

estrogen and supplementing such dissections by microscopic study of nodules which they observed in these organs of the mice.

In our present investigations, only those mice are included whose organs have been studied microscopically. To make possible the statistical treatment of our data, it was necessary to attach grades designating the state of proliferation in the mammary glands of the individual mice, as follows: Grade 0 was given when ducts were so uncommon that none were seen in the large majority of sections; grade 1 signified a resting mammary gland consisting mainly of scattered ducts, to which a few isolated acini or, rarely and exceptionally, small groups of acini may have been attached. A mammary gland in which larger groups or lobules of acini were found received grade 2. In animals with this grade, some acini were often observed in the process of secretion, while some other ducts or acini showed slight proliferative processes. When the latter processes became more marked and led to the production of precancerous states, grade 3 was given. If carcinoma had developed, the gland was placed in grade 4. There were intermediate conditions of the mammary gland which were designated by intermediate grades. We distinguish thus, in the main, three stages in the development of mammary gland carcinoma: (1) The period or phase of resistance to growth characterized by grade 1 (RP). In this condition the mammary gland is essentially in a resting stage, with the possible exception of slight cyclic changes, from which the gland again returns to a resting stage. Single injections or a small number of injections of estrogen do not usually lead to marked growth; at best they result in only transitory growth. (2) The phase of preparatory growth (PP), comprising grades 2 and 3 and those intermediate between 2 and 3. This period is characterized by definite and more permanent growth, which increases with increasing action of hormones. The condition of precancerous growth (grade 3) forms the transition to the end stage of this series, cancerous growth (CP), to which grade 4 has been attached.

NUMBERS OF MICE USED

Table 1 shows the numbers of mice from the different strains which served in these investigations. In order to increase the number of our data we have included also the majority of the mice into which anterior lobes of hypophysial glands had been transplanted in experiments on which we have already reported. These mice behaved very similarly to those which received large doses of estrogen; this applies especially to animals belonging to strain A, which had served largely in the previous investigations. Table 1 shows that in these investigations altogether 756 mice were used; the important organs of all of these were examined microscopically; this includes 49 mice into which anterior lobes of hypophyses had been transplanted or which served as controls for the

latter. Of these 756 mice, 321 were controls, and in 435 the mammary glands had been stimulated experimentally. Among the different strains, the number of mice available in strain CBA and the number in strain New Buffalo were rather small while the number of strain A mice was the largest.

ESTROGEN TREATMENT

The doses of estrogen which were injected varied between 100 and 200 rat units of estrogen dissolved in oil, usually one subcutaneous injection being given weekly. Some mice received as much as 200 rat units weekly; others received once weekly 100 rat units of estrogen in oil and 50 rat units dissolved in water. Doses of 100 or more rat units of estrogen dissolved in oil were large doses. Medium doses were amounts of estrogen varying between 10 and 50 rat units dissolved in water, injected as a rule five or six times weekly. Other mice

TABLE 1.—*Number of Mice Used in the Various Strains*

	Strains							
	C57	CBA	Old Buffalo	New Buffalo	A	D	C3H	Total
Normal controls	47	17	25	16	69	34	18	226
Ovariectomized controls	22	2	39	17	4	6	5	95
Mice given medium and small doses of estrogen	16	0	16	6	43	20	28	129
Mice given large doses of estrogen.	66	33	14	19	72	33	69	306
Total	151	52	94	58	188	93	120	756
Mice receiving anterior hypophysial transplants	7	3	0	0	27	2	10	49

received small doses of estrogen—amounts of less than 10 rat units dissolved in water.

OBSERVATIONS

We shall now analyze our findings in the various strains of mice from the stated point of view as far as the material at our disposal makes such an analysis possible.

Table 2 summarizes our observations in the various strains which we examined. In table 3 the figures for the low tumor strains, C_{57} and CBA, and those for the Buffalo strains and for the high tumor strains, C_3H and D, are combined. Strain A, which is intermediate between the Buffalo and the high tumor strains, C_3H and D, and from which the greatest number of mice were available, is considered separately.

Strain C_{57}.—In normal male mice of this strain the average grade of the mammary gland was below 1, a condition observed in all strains. In all virgin female control mice of this strain up to the age of 22 months, the stage of resistance was found. The mammary gland consisted of scattered ducts, with which only rarely a few acini could be seen. No definite preparatory growth period

was observed in these mice under the influence of endogenous estrogen. In 1 of 3 breeding mice, 15 months old, a few small groups of acini developed in the mammary gland (grade 1.25). In former observations we found atrophic as well as some better developed ducts and only occasionally a few isolated acini or very small groups of acini; very rarely in some of these acini or in small ducts a slight secretion was noticed. In ovariectomized mice the grades ranged between 0 and 1.

Among mice receiving injections of small doses of estrogen a preparatory period was observed in 3 females, aged between 21 and 25 months (grades 1.25, 2, 2.25), but in other mice at this age the mammary gland was as yet quite inactive. Several mice from 20 to 25 months old remained in the resting stage.

With medium doses (20 rat units) of estrogen dissolved in water, a beginning preparatory period (grade 1.25) was noted in 2 female mice, 10 and 18 months old, while in 3 mice, 13.5 to 16.5 months old, long-continued injections of 50 rat units of estrogen dissolved in water were without effect; likewise, a number of mice, 9, 10, 14 and 17 months old, were still in the resting stage.

With large doses of estrogen, a preparatory growth period (grade 1.75) was noted in 2 male mice, 3 and 4 months old, which had received 200 rat units, and it was likewise found in 2 male mice, 12 and 13 months old, which had been given 100 rat units (grade 1.75 and 2). Other young mice in this series, excepting the 2 mentioned, did not show a preparatory growth period. In general, the larger doses were more effective in eliciting the preparatory growth than the smaller ones. Likewise, among female virgin mice given 100 rat units a preparatory growth was found in 2 mice, 11.5 and 12.5 months old (grades 1.75, 2.25), and also in a 5.5 month old mouse; but a number of 8.5 and 9.5 month old mice did not pass out of the period of resistance. There occurred, then, a definite acceleration of the growth period under the influence of exogenous estrogen; as a rule, it affected only middle-aged mice. The highest grade attained was 2.25; a further intensification of the growth into the precancerous stage was not accomplished. Formation of lobules and of large acinous cells, in which mitotic proliferation occurred, was the highest degree of growth that was reached under these conditions in the C_{57} strain.

Strain CBA.—In control mice of this strain there was likewise a long period of resistance; even in mice as old as 26 and 31 months grade 1 was found. As in all other strains, so also in strain CBA in male and in ovariectomized mice the grades were 1 or less. Likewise in breeding mice the grade was 1, except in 1 mouse, in which a precancerous condition had developed, and except also in those which were examined within one month after the termination of pregnancy. It is possible that if a larger number of control mice in strains C_{57} and CBA had been studied, animals with a more extensive growth of the mammary gland might have been encountered. Among the control mice included in this series, tumors developed in 2 animals.

Injections of 200 rat units of estrogen could induce in male and in female mice the development of a preparatory growth as early as two months after the beginning of the injections in 2.5 month old mice. In a male mouse 7.5 months old an almost precancerous condition was reached (grade 2.75).

In ovariectomized mice given 150 rat units of estrogen the preparatory growth period was first observed at the age of 4 months, after injections had continued for two months, and similar changes were found up to the age of 8 months, which was the latest period at which these mice were examined. Therefore, in mice given somewhat smaller doses than 200 rat units per week the preparatory growth period set in a little later. However, in no case did the proliferation proceed

TABLE 2.—*Duration of Period of Resistance and of Periods of Preparatory and Cancerous Growth, and Grades of Growth, in Control and Treated Mice Belonging to Different Strains* *

Strain	Period of Resistance			Preparatory Growth Period				Cancerous Growth Period		
	Mice	Length of Period, Mo.	Average Number of Months	Mice	Length of Period, Mo.	Average Number of Months	Average Grade	Mice	Length of Period, Mo.	Average Number of Months
Strain C57										
Controls:										
Nonbreeding	41	1-22	7.7	..	.....	....	....	..	.....	....
Breeding	2	2-15	8.5	4	12.5-15	13.5	1.25	..	.....	....
Mice given injections:										
Medium and small doses of estrogen	11	5-25	15	5	10-25	20	1.55	..	.....	....
Large doses of estrogen	29	1.5-10.25	5.8	12	3.5-13	8.8	1.72	..	.....	....
Large doses of estrogen (including estrogen plus progesterone [proluton])	31	1.5-12	6	35	3.5-16.5	10.3	1.7	..	.....	....
Strain CBA										
Controls:										
Nonbreeding	10	2.25-31	20.8	1	11.5	11.5	1.25 } 2.12	..	.....	....
Breeding	3	15-20	16.7	1	28	28	3 }	2	17-26	21.5
Mice given injections:										
Medium and small doses of estrogen	..	.....	....	..	.....	....	....	..	.....	....
Large doses of estrogen	5	7.5-8	3.7	28	2.5-13.5	6.6	1.94	..	.....	....
Strain Old Buffalo										
Controls:										
Nonbreeding	14	2.25-29	13.4	4	10-17	13.3	1.43 } 1.54	2	19-26	22.5
Breeding	2	10-13	12	3	12.5-36	24.8	1.66 }	..	.....	....
Mice given injections:										
Medium and small doses of estrogen	11	10-14	13	5	12.5-25	15.5	2.25	..	.....	....
Large doses of estrogen	..	.....	....	10	3.25-14.5	7.4	2.2	4	12-13.5	12.6
Strain New Buffalo										
Controls:										
Nonbreeding										

Strain New Buffalo										
Controls:										
Nonbreeding	2	28	28	3	13-18	14.7	1.5 } 1.75	4	13-21	18
Breeding	1	11.5	11.5	2	11.5-18	14.7	2.12 }	4	15-23	20
Mice given injections:										
Medium and small doses of estrogen	..	.....	....	5	13-20	16	2.25	1	24	24
Large doses of estrogen	6	5-11.25	7.25	9	3.5-12	7.2	2	4	7.5-17	14.6
Strain A										
Controls:										
Nonbreeding	47	2-24.5	8.5	1	23	23	3 } 1.84	3	13.5-17	15.2
Breeding	8	10-14	11.8	7	10-15	12.3	1.68 }	3	10-12	11.3
Mice given injections:										
Medium and small doses of estrogen	19	3-21	12.5	8	10-19	16.3	1.7	16	11-20	16
Large doses of estrogen (including estrogen plus progesterone [proluton] or anterior hypophysial implants)	28	3-14	6.7	22	5.5-12.5	9.9	1.98	22	7-13.5	9.6
Large doses of estrogen alone	26	3-4	6.4	9	5.5-12	8.6	1.4	10	7.75-13.5	9.8
Strain D										
Controls:										
Nonbreeding	7	1.5-16	7.6	5	10-15	12.2	1.37 } 1.62	7	13-22	17
Breeding	1	11.5	11.5	2	10.14	12.75	2.25 }	12	9-22	13
Mice given injections:										
Medium and small doses of estrogen	3	2-8.25	4.25	6	8-17.5	11.8	2.9	11	11-21.5	16
Large doses of estrogen	13	1.5-13.25	4.7	8	3.5-21.5	9.4	2	12	6.5-11.25	9.1
Strain C3H										
Controls:										
Nonbreeding	..	.....	....	1	17	17	2.25 } 2.0	3	10-20	14.7
Breeding	..	.....	....	3	10-15	12.9	1.92 }	11	8-15	11.8
Mice given injections:										
Medium and small doses of estrogen	5	10.75-17	13.8	8	4.75-19	13.8	1.96	15	6-17	12.8
Large doses of estrogen	10	2-21.7	7	30	3-12	5.7	1.93	29	4.5-18	9.1

* The table includes mice receiving anterior hypophysial implants.

TABLE 3.—*Duration of Period of Resistance and of Periods of Preparatory and Cancerous Growth, and Grades of Growth, in Control and Treated Mice Belonging to Combinations of Strains*

Strain	Period of Resistance			Preparatory Growth Period				Cancerous Growth Period		
	Mice	Length of Period, Mo.	Average Number of Months	Mice	Length of Period, Mo.	Average Number of Months	Average Grade	Mice	Length of Period, Mo.	Average Number of Months
Strains C57 + CBA										
Controls:										
Nonbreeding	51	1-31	10.3	1	11.5	11.5	1.22 } 1.54	2	17-26	21.5
Breeding	5	2-20	14	5	12.5-28	16.4	7.6 }	..	.....	....
Mice given injections:										
Medium and small doses of estrogen	11	5-25	15	5	5-25	20	1.55	..	.....	....
Large doses of estrogen (including estrogen plus progesterone [proluton] or anterior hypophysial implants)	36	1.5-12	5.7	63	2.5-16.5	8.65	1.81	..	.....	....
Strains Old Buffalo + New Buffalo										
Controls:										
Nonbreeding	16	2.25-29	15.2	7	10-18	13.6	1.5 } 1.64	6	13-26	19.5 } 19.3
Breeding	3	10-13	11.7	5	10-36	20.7	1.84 }	3	15-23	19 }
Mice given injections:										
Medium and small doses of estrogen	11	10-14	13	10	12.5-25	15.7	2.25	1	24	24
Large doses of estrogen	6	5-11.25	7.25	19	3.25-14.5	7.3	2.1	8	7.5-17	13.5
Strain A										
Controls:										
Nonbreeding	47	2-24.5	8.5	1	23	23	3 } 1.84	3	13.5-17	15.2 } 13.25
Breeding	8	10-14	11.8	7	10-15	12.3	1.68 }	3	10-12	11.3 }
Mice given injections:										
Medium and small doses of estrogen	19	3-21	12.5	8	10-19	16.3	1.7	16	11-20	16
Large doses of estrogen (including estrogen plus progesterone [proluton] or anterior hypophysial implants)	28	3-14	6.6	22	5.5-12.5	9.9	1.98	22	7-13.3	9.6
Large doses of estrogen alone	26	3-4	6.4	9	5.5-12	8.6	1.4	10	7.75-13.5	9.8
Strains C3H + D										
Controls:										
Nonbreeding	7	1.5-16	7.5	6	10-17	13	1.5 } 1.8	10	10-22	16.3 } 13.6
Breeding	1	11.5	11.5	5	10-15	12.7	2.1 }	23	9-22	12.4 }
Mice given injections:										
Medium and small doses of estrogen	8	2-17	10.2	14	4.75-19	12.9	2.4	36	6-21.5	14.1
Large doses of estrogen	23	1.5-21.7	6.7	28	3-21.5	6	1.99	41	4.5-18	9.1

to a typical precancerous stage, although lobule formation, mitotic proliferation and secretion could be observed. In the large majority of all the CBA mice given large doses of estrogen, the preparatory growth period set in at an average age of 6.6 months.

On the whole, the effect of large doses of estrogen on the preparatory growth of the mammary gland was noted earlier and more uniformly in CBA than in C_{57} mice. The mammary gland tissue in the CBA strain seems to be somewhat more responsive to hormone stimulation than that in the C_{57} strain.

Strain Old Buffalo.—If we exclude the mice which served as controls to those which received anterior hypophysial transplants, the resistance to proliferation was very strong in the control mice. In the large majority of the nonbreeding mice only ducts were seen, but in 2 mice belonging to this group tumors developed at a late stage of life. However, the proportion of nonbreeding mice which entered the preparatory growth period was greater in the Old Buffalo strain than in the C_{57} and CBA strains, and the degree of proliferation was likewise greater. As in the other strains, the proportion of mice which reached the preparatory growth phase was much greater in the breeding than in the nonbreeding group. In ovariectomized nonbreeding as well as breeding mice only ducts were seen, and as a rule they were present only in small numbers. Ovariectomy in control mice prevented the appearance of a preparatory growth period for at least six weeks.

In mice in which medium doses of estrogen were injected daily, tumors did not develop, but a considerable amount of very active mammary gland tissue was produced after twelve and twenty-four and one half months of injections (grades 3, 2.75). The preparatory growth period was therefore far advanced in these mice. In 13 and 14 month old mice which had been given daily injections of smaller quantities of estrogen, or combinations of estrogen and lutein preparations,[7] or anterior hypophysial extract, for periods of eleven to twelve months, the mammary gland tissue showed a development similar to that seen in controls. The large majority of the mice were in the stage of resistance; in only 1 mouse had a preparatory growth set in.

In male as well as in virgin female mice given 100 rat units of estrogen weekly, a definite acceleration of the preparatory growth period took place. In 4 of 6 mice which were from 12 to 14.5 months old, the stage of tumor growth had been reached; the 2 other mice still remained in the preparatory period of growth, which led to a marked development of lobules. These tumor mice had passed through a preparatory growth phase before they entered the cancer stage, and the grade attaching to the first-named period should correspondingly be increased.

While in CBA mice under the influence of large amounts of estrogen the preparatory growth period sets in about as readily as in Old Buffalo mice, in the latter the growth proceeded further—it reached a precancerous stage; in CBA and C_{57} mice it ceased some time before this stage had been attained. These observations agree with our earlier findings to the effect that some of the middle-aged Old Buffalo virgin as well as breeding mice were found in various stages of the preparatory growth period and some secretion was noted in various acini of the mammary gland.

Strain New Buffalo.—In some virgin control mice mammary gland tumors developed at the age of from 13 to 21 months, while in still older mice, aged 28 months, the mammary gland was still in the stage of resistance (grade 1).

7. The preparations used were a corpus luteum extract prepared in our laboratory and a corpus luteum extract prepared by the Schering Corporation (proluton).

In the Old Buffalo and New Buffalo, as well as in the other strains, the mammary gland tissue of mice with breast tumors showed, as a rule, also outside the tumor area a marked development of acinous tissue, often associated with lobule formation, hypertrophy, mitotic proliferation and secretion, which were more marked than in non-tumor-bearing mice. However, occasionally there was a tumor-bearing mouse in which the greater part of the remaining mammary gland was still in the phase of resistance. The findings varied in older mice which died without mammary gland tumors. The conditions observed here ranged from the presence of ducts, with or without a few acini (grade 1) in some instances, to the development of a somewhat greater number of acini, some of which showed secretion (grade 1.25 in 13 month old mice), and further to the formation of lobules, in which some acini were lined with large cells and secretion was evident.

In 4 of the breeding mice, 15 to 23 months old, tumors arose, and precancerous tissue formed in some areas in the noncancerous mammary gland tissue. In others, preparatory growth was found. As usual, ovariectomy tended to reduce the activity of the mammary glands.

These observations suggest that the period of resistance in New Buffalo control mice was shorter and the preparatory period, on a whole, set in more readily than in Old Buffalo, CBA and C_{57} mice. The same applies to the transition from preparatory growth to cancer growth.

In female mice which had been given, twice weekly, injections of medium doses of estrogen from the age of 2 weeks on, the grades 2 and 2.25 had been reached at the age of from 13 to 20 months; tumors did not develop in these animals. In a female virgin mouse 24 months old, which had been given small doses of estrogen for twenty-three months, a tumor developed; in a 20 month old mouse which had been treated similarly for nineteen months, the grade was 2.75, and in a mouse 12 months old which had been given 1 to 10 rat units of estrogen for ten and one half months the grade was 2.

These experiments, in which medium or small doses of estrogen were injected, confirm therefore the findings in control mice; they indicate that in old mice of the New Buffalo strain cancer of the mammary gland may develop and that this stage is preceded by a preparatory growth period which extends from middle age to the early period of old age.

With injections of large doses of estrogen, the preparatory growth period was accelerated in female virgin mice in the same way as it was in the Old Buffalo strain; in some mice 5.5 months old or older, the grade rose to 2. Accordingly, also, the cancerous growth set in earlier and tumors appeared at the age of 7.5, 14, 17 and 20 months. Similarly, in male mice in which the injections of large doses of estrogen had begun at the age of 1 and 2 weeks and whose age at the time of death varied between 5 and 11.5 months, the grades 1.75 and 2.75 were attained in mice 7.5 months old and older. The preparatory growth period had therefore begun at an earlier time of life, but in a number of these mice the period of tumor growth had not yet been reached when the mice died or were killed.

Strain A.—The large majority of the nonbreeding control mice were found in the stage of resistance; this applies even to mice 24.5 months old. In only 1 mouse, 23 months old, was the preparatory growth period observed, but it had progressed already to the precancerous stage, and in 3 middle-aged mice the cancerous phase had been reached. Evidently in nonbreeding mice of strain A the mammary gland is relatively inert and the large majority of the animals remain in the stage of resistance, but if they enter the growth phase of preparatory growth, they seem to pass through the latter readily until they enter the cancerous stage.

The proportion of breeding mice which entered the period of preparatory growth was much greater than that of nonbreeding mice, and the age at which this process occurred was earlier (12.3 months), but the average grade of proliferation was not high. Likewise, the tumor phase was reached earlier in the breeding mice. The average age of the preparatory growth phase and that of the cancerous phase were similar, which may perhaps be taken as an indication that the transition from the former to the latter phase occurs rather rapidly.

With the smallest dose of estrogen, conditions were similar to those seen in the controls. A preparatory period was found in only a single mouse, 14 months old (grade 2.25). As in control mice, the preparatory period may therefore set in late in life, when in other mice the tumor period has already been reached. A tumor developed in a middle-aged mouse.

With the lower range of medium doses (10-30 rat units) of estrogen, many tumors developed, even in nonbreeding mice, at the age of 11 to 20 months. The large majority of these mice had been given injections for eleven months or longer. In other mice of this age a preparatory growth period was observed, and in some this had progressed to a precancerous stage; it may be assumed that in these animals tumors would have developed before long. In younger mice there was a state of resistance. These mice also behaved similarly, therefore, to control mice, except that the preparatory growth changed more readily into cancerous growth.

When 50 rats units of estrogen was injected, a not very active preparatory growth was observed in middle-aged as well as in slightly older mice (grades 1.25, 1.75); in these animals, males as well as females, the preparatory growth had not yet progressed to the tumor stage. In others, up to the age of 21 months there was still a state of resistance in the mammary gland.

The proportion of mice given large doses of estrogen which entered the phase of preparatory growth was greater than the proportion of those given smaller doses or than the proportion of the nontreated control mice. Also, the age at which they entered the preparatory growth period was earlier, and likewise the transition to the phase of cancerous growth occurred earlier. Hence both the preparatory growth period and the tumor period were accelerated by large doses of estrogen, although even with large doses some middle-aged mice were still found in the stage of resistance. While, on the average, the preparatory growth period was reached somewhat later in the A mice given large doses of estrogen than in other strains, it seems that these animals passed rather quickly through this period to enter the phase of cancerous growth. If we exclude the mice which received anterior hypophysial transplants, the grade of proliferation reached in the A strain which had entered the preparatory growth phase was, on the average, low.

Strain D.—The condition of the mammary gland of virgin control D mice did not differ to a marked degree from that of the low tumor strain mice during the first four months of life. However, in female virgin D mice between the ages of 13 and 22 months mammary gland carcinoma developed. The tumors, therefore, appeared much earlier in D mice than in CBA and in Old Buffalo nonbreeding mice, in which the earliest spontaneous tumors were noted at the age of 19 months and among which even some 25 and 29 month old mice were found without tumors. Concerning the preparatory growth period in these control mice, data as to the condition of the mammary gland between the ages of 4 and 13 months are lacking, but such a growth was observed in middle-aged mice; and the fact that in the majority of the nonbreeding mice affected by cancer the mam-

mary gland outside the area of transformation showed marked development indicates that a preparatory growth period had preceded tumor formation in these mice. The same observation was made in breeding mice. In only 1 animal, 11.5 months old, was the mammary gland found in the phase of resistance. As to the mammary glands of older breeding mice, a preparatory growth was found in 2 mice, while in the large majority tumors developed. The earliest age at which a tumor was noted was 9 months. Therefore, tumors appeared here somewhat earlier than in virgin mice. In the large majority of this group of tumor mice, again, the epithelial tissue outside the tumor area showed a condition corresponding to a preparatory growth period. That such a period precedes the development of cancer also in D mice is indicated by the fact that in some nonbreeding as well as breeding controls of this strain, which had received anterior hypophysial transplants, a preparatory growth period was actually observed preceding the appearance of tumors, and furthermore the preparatory growth of the mammary gland was more intense in these D mice than in A mice.

In breeding as well as nonbreeding mice ovariectomized at the age of 2 or 5.5 months and examined at the age of from 5 to 8 months, the grades were 1 and 0.75; tumors had not yet appeared at the age of 13 months. This agrees with our earlier experiments, as well as with those of Cori, which have shown that in mice ovariectomized at the age of 2 months tumors do not appear even at late periods of life. The effect of removal of the ovaries under these conditions is therefore much stronger than the effect of prevention of breeding; besides, it seems that the mammary glands of ovariectomized mice are very similar, irrespective of whether these mice belong to high or to low tumor rate strains.

In D mice given small or medium doses of estrogen a preparatory growth period appeared at the age of from 8 to 17.5 months—on the average, at the age of 11.8 months—and it progressed in some animals to grade 3; it would, therefore, in all probability, within a short time have been transformed into cancerous growth. Tumors were found between the ages of 11 and 21.5 months, an age range only slightly lower than that of the control nonbreeding tumor mice. It is possible that the preparatory growth phase which preceded tumor growth actually set in at a somewhat earlier period than that observed in these mice. We may conclude that in D mice given medium-sized doses of estrogen the growth of the mammary gland was intensified and tumor development accelerated as compared with the nonbreeding controls but that these effects did not exceed noticeably those observed in breeding controls.

In D mice given large doses of estrogen the preparatory growth period appeared earlier than in mice given smaller doses of estrogen or in control mice. In animals given 200 rat units of estrogen it was noted at the early age of 3.5 and 4.5 months, while previous to that time the mice were still in the period of resistance. In mice which had been given 100 rat units of estrogen such a preparatory growth period could be noted at the age of 21.5 months, but in a considerable number of young mice, and even in some middle-aged mice the mammary gland was found in a state of resistance at the time of examination. Tumors appeared in a large number of these mice at an average age which was lower than the average age in Buffalo mice and somewhat lower than that noted in A mice.

Strain C₃H.—In 3 of 4 nonbreeding control mice 10 to 20 months old, mammary gland carcinoma developed. The fourth mouse, 17 months old, was found in the preparatory growth period, with grade 2.25; a tumor would in all probability have developed in this animal in the course of time.

Tumors developed in the large majority of the breeding control mice between the ages of 8 and 15 months, i. e., at an average of 11.8 months; the remaining 3 mice, varying in age between 10 and 15 months, were found in the preparatory growth phase. The average age at which the preparatory growth period was found in breeding control mice (12.75 months) was very similar in strains D, C$_3$H and A; it was lower than in other strains. Since tumors appear at a relatively early age in these breeding controls, we must assume that here, as in A and D mice, the mammary gland passes rather quickly through this phase to enter the phase of cancer growth and that thus an apparent overlapping of these two periods may result.

Among male mice given medium doses of estrogen, tumors developed in 6, while 11 were free of tumors. The age at which the tumors appeared in males was about the same as that in female breeding control mice, but the proportion of mice affected was somewhat smaller among the former than among the latter. As usual, the mammary gland outside the tumor area showed in all cases marked proliferation (grade 3). As to the 11 mice without tumors, 5 were still in the stage of resistance, while 6 were in various stages of the preparatory growth period. Of 11 female nonbreeding mice, tumors developed in 9, beginning at the age of 8 months; again, in these mice the mammary gland outside the tumor area was strongly proliferating, with grade 3 in all cases. In 2 mice, 4.75 months old (grade 1.25) and 9.5 months old (grade 2.75), the mammary gland was as yet in the preparatory growth period, which set in, therefore, at a time preceding the tumor period. In both males and females the injections were begun at about the age of 2 weeks, but in this instance the effect was greater in the female than in the male mice.

Administration of large doses of estrogen to male C$_3$H mice accelerated the appearance of tumors. In 5 males given 200 rat units of estrogen, beginning at the age of 2 weeks, tumors formed between the age of 4 and 8.5 months. In a mouse 5.5 months old the mammary gland was still in the preparatory growth stage, with grade 2.25. In 9 males in which injections of 150 rat units of estrogen began at the age of from 2 to 5 weeks, tumors appeared at the age of 6, 8.5, 11 and 18 months. A mouse treated for six months, which was 7 months old at the time of examination, was in the preparatory growth period (grade 3); it would probably soon have entered the period of cancerous growth.

However, in other mice, 5.5 and 6.5 months old, the mammary glands were as yet in the phase of resistance. In a male mouse given 100 rat units of estrogen a tumor developed at the age of 7.75 months. As noted in so many other cases, the mammary gland outside the tumor area was in an advanced stage of proliferation.

Also, in female mice given 150 rat units of estrogen tumors appeared from the age of 8 months on; therefore, at a somewhat earlier age than in the control mice. In a somewhat younger, 5.5 month old, mouse the mammary gland had not passed the preparatory period of growth (grade 1.75), and in 2 older mice as well, in which no tumors had developed at the time of examination, the mammary gland was in this preparatory phase. In animals ovariectomized at the age of from 1 to 2 months and given 150 rat units of estrogen, mammary gland carcinoma appeared at the age of 6, 7 and 7.5 months, but in other mice in this age range and in younger mice from the age of 3 months on, the mammary gland was still in the stage of preparatory growth. The latter stage began, therefore, in this case several months before the appearance of cancer. From the age of 8.5 months on, all mice had mammary gland carcinoma. Among 5 female mice from 6 to 9 months old and treated with 100 rat units of estrogen, tumors developed in 4.

It may than be concluded that in strain C_3H, which posseses a strong hereditary tendency to the development of tumors, the preparatory growth period sets in relatively early in life and then passes rather soon into the cancerous growth stage; however, this transitional preparatory period may last several months and perhaps even longer in some instances.

COMMENT

Our main aim in these investigations has been the determination of the relation between the formation of carcinoma in the mammary gland and the growth processes in this organ which precede the change of the normal into cancerous tissue and, in particular, the determination as to whether there is a correspondence between the readiness with which carcinoma develops in a certain strain and the readiness and intensity of the development of growth processes in the mammary gland, which eventuate in the cancerous transformation. Our previous microscopic studies of the changes taking place in this organ in various strains of mice, together with experimental studies which have been carried out during the past eight years, have shown that such a preparatory growth period exists, and they have indicated that it bears a certain relation to the inherited tendency toward the development of mammary gland carcinoma; likewise, the findings of Gardner, Diddle, Smith, Strong and Allen [5] agree with this conclusion. In no instance did we observe a direct transition from the resistant phase (RP) to the cancerous phase (CP). There is always, as far as we know, a preparatory growth phase interposed between RP and CP, which becomes more and more intensified in the course of time, until a precancerous condition, and ultimately a cancerous state, results. At least this is true in the case of hormonal cancer. It is possible, furthermore, to observe in the majority of mice in which mammary gland carcinoma develops, more or less advanced growth processes in the remaining gland tissue, and it is on the basis of these growth processes that cancer develops. These must be interpreted as representing the preparatory growth stage in the mammary gland, different parts of which have developed with unequal rapidity, so that one has reached the stage of CP, while others may be closely following in the progression from RP to CP. In some cases it may happen, however, that the progressive changes may be limited to a smaller area of the gland and that the other areas remain relatively quiescent for a longer period.

We now wished to determine the relations of the various developments as much as possible in a quantitative manner by means of a statistical analysis of our observations. The latter are based on the microscopic study of the mammary glands, ovaries and certain other sex organs of 756 mice. While this may appear as a large amount of

material, it is not yet sufficient to answer in a definite form all the questions which may be asked, because the presence of various variable factors introduces uncertainties. Some of these variable factors might be eliminated by making further subdivisions in each strain of mice, for instance, in accordance with the dose of estrogen which has been administered or with the growth phase reached in the mammary gland. But in making these subdivisions the number of mice which comprise each subdivision becomes so small that other variable factors, which often are unknown, assume prominence and invalidate our statistical conclusions. If, on the other hand, in order to obviate the last-named difficulty various smaller groups are combined, we bring into one division groups which are heterogeneous in certain important respects, as happens if we combine animals treated with medium and small doses of estrogen. Such differences in dosage may cause differences in the effects, which remain hidden. The action of unknown factors is also made evident by the observation that the mammary glands of different mice which belong to the same closely inbred strain and should possess, therefore, the same hereditary tendency toward cancer and which have received approximately the same amount of experimental stimulation differ greatly in the readiness with which they undergo cancerous transformation. Secondary and variable factors must be responsible also for the variations in the behavior of different, and often of neighboring, areas in the mammary gland of the same animal. In view of the presence of so many interfering conditions, not all of our conclusions can be considered as definite; some of them serve at present merely as a suggestion of the direction in which investigations should proceed in the continuation and extension of experiments of this kind.

In a general way we may interpret the data given in the tables as indicating that in the course of life the mammary gland of each mouse has a tendency to undergo progressive alterations in which the small ducts or acini, besides manifesting certain rhythmic processes during the sexual cycle or undergoing growth and metabolic alterations during pregnancy and lactation, conditions which are followed by an approximate return to the original state, gradually pass from a stage of relative rest and inactivity (RP), in which the gland consists mainly of some large and many small ducts, perhaps with the addition of a few isolated acini or exceptional small groups of acini, to a phase of preparatory growth (PP), when more numerous and larger groups of acini or whole lobules of gland tissue are produced, in which usually secretion and proliferative processes are associated within the same gland. Gradually these processes are intensified in certain places, the cells reach a larger than usual size, and inequalities in the size of cells and nuclei, in the amount of chromatin and in the character of the nucleoli may appear.

These localized responses to stimuli, acting over relatively long periods, may lead to abnormal growth processes in the ducts and acini, in which often a certain coiling is a characteristic early deviation from the normal. There seems to be a tendency also toward increased cell movements, as indicated by the more frequently observed penetration of acinus or duct cells into the viscous or solid material which fills the cavities of these acini or ducts, and thus by way of precancerous states the tissue passes, here and there, into the stage of an irreversible cancerous growth (CP). It usually happens that in one place in the glands the process is more advanced than in others, but if the stimulation continues, the same process takes place also in other places; multiple areas of precancerous and cancerous proliferation develop, and even larger areas of the gland begin to participate in this active growth, which ultimately would lead to a generalized CP, an extreme state, which during the life span of these animals is actually never reached. In general, among all mice there is a tendency for the mammary gland to change from RP to PP and to CP in the course of the life of the individual animals. In female control mice this takes place under the influence of endogenous ovarian hormones, given off during normal life, but it is accelerated and intensified by adding exogenous hormones.

Different strains differ greatly in the average rapidity with which they pass from RP to PP and CP. In strain C_{57}, only some breeding mice moved from RP to PP; they entered PP rather late in life and did not reach a high grade of proliferation, so that they did not approach the transition stage to CP. In none of the nonbreeding mice in strain C_{57} which we studied was PP reached. With larger doses of estrogen, this process was accelerated, but still CP was not attained. Therefore, in strain C_{57} the endogenous ovarian hormone produces a very slow and incomplete PP, no mouse arriving at the borderline that separates PP from CP. In CBA this process was somewhat accelerated; the number of mice entering PP was greater and the degree of proliferation which they reached in PP was higher, but only a very small percentage of mice passed into the CP stage. In Old Buffalo and still more so in New Buffalo the transition from RP to PP took place more quickly and, likewise, the progress in PP was more rapid than it was in C_{57} and CBA; the number of mice which reached CP was increased. The transition from RP to PP and from PP to CP was still more accelerated in the high tumor rate strains D and C_3H; the number of mice that entered PP was greater, they passed more rapidly through this phase and a greater number of them reached CP than in the other strains. Strain A did not quite approach the intensity of the growth processes of the high tumor rate strains, and it entered CP more

slowly under the influence of endogenous ovarian hormones than did strains D and C_3H.

An important point in these findings is, then, the fact that in C_{57} mice the lack of cancer formation seems not to depend on the inability of these mice to cross the border from PP to CP by way of a precancerous state but on their inability to produce enough growth energy to pass completely through the preparatory growth phase, which is a necessary prerequisite for the cancerous transformation. In general, in high cancer rate strains the average age in PP was relatively increased and the average grade of proliferative intensity lowered by the fact that mice in which conditions were favorable entered CP at a relatively early stage and mice in which unknown factors tended to weaken the growth of the mammary gland were left in PP.

The number of mice which were found in PP depended at least on two factors: (1) The readiness of the animals to pass from RP to PP. The stronger this factor, the greater will be the number of mice in PP and the more rapidly will this stage be reached. (2) The readiness with which the change from PP to CP occurs. The more readily this transformation takes place, the smaller will be the number of mice left in PP. The number and the average age of mice in PP depend, therefore, on complex conditions, and they are not an unequivocal indication of the growth energy characteristic of the various strains of mice. A more adequate evaluation of this factor can be reached only if we combine mice in PP and in CP and compare this combined number with the number of mice left in RP. However, the proportion of mice found in RP and PP depends, in addition, on the number and age of the mice which were used for observation in RP. The consideration of the age of the latter is therefore important. The tumor rate in various strains of mice depends not only on the number of animals which pass from PP to CP and on the rapidity of this process but also on the readiness with which they pass from RP to PP and on the rate at which they pass through the stage of preparatory growth.

Parallel to the increase in the cancer rate in various strains of mice there takes place a lowering of the average length of the preparatory growth phase; likewise, there occurs a lowering in the mammary gland cancer age in the various strains under the influence of breeding, of large doses of estrogen and of anterior hypophysial transplants, and this is accompanied by a lowering of the PP age in these same strains of mice.

The lowering of the tumor age in breeding mice is shown in table 4. This effect is lacking in the Old Buffalo strain, and it is relatively slight in the New Buffalo strain, in correspondence with the fact that in the Buffalo strains also the tumor incidence is increased by breeding to a lesser extent than in the other strains. The difference

is most marked in the A strain and marked also in the D and C_3H strains, in accordance with the great effect of breeding on the tumor incidence in these mice. No statement can be made as to strain CBA in this respect, because only one tumor has been observed in a CBA nonbreeding mouse, and in this mouse the tumor appeared late in life. In tables 2 and 3, in which only a restricted number of tumor mice have been used, the relation between the average tumor ages of the breeding and the nonbreeding mice is similar in the various strains to that seen in table 4, although, as should be expected, the figures are not exactly the same as those obtained in that table.

The conclusion as to the general relation of PP to CP is strengthened also by the observation that a certain parallelism exists between the directions in which several factors influence the average age of mice in these two phases. The strain characteristics and the doses of estrogen

TABLE 4.—*Average Age of Control Mice with Tumors (Including All Mice Thus Far Observed)*

Strain	Nonbreeding Mice			Breeding Mice		
	Number	Age, Mo.	Average Age, Mo.	Number	Age, Mo.	Average Age, Mo.
CBA	1	24	24	12	9-28	15.3
Old Buffalo	8	11-23	17.1	6	13-23	17.5
New Buffalo	17	9-23	18.5	35	6-25	16.4
A	6	15-22	17.7	157	5-20	11.2
D	51	8-23	15.5	246	5-22	10.3
C3H	27	7-22	14.3	179	5-18	9.8
Old Buffalo + New Buffalo	25	9-23	18	41	6-25	16.5
D + C3H	78	7-23	15.1	425	5-22	10.1

which cause a lowering of the age in CP also cause a lowering in PP, as shown in tables 3 and 4. It is due to variable factors that, although in accordance with expectation in the majority of instances the average age in CP is higher than that in PP, there are a number of instances in which the average ages of PP and CP are similar. Such a similarity would obtain if among the mice in the PP phase there were some which happened to be older than the average without having entered as yet the cancerous phase. Other errors are introduced by the fact that our data are incomplete in various respects. Thus it happens that, among virgin control mice of strain D, animals between the ages of 4 and 10 months have not been examined; this makes the average age of PP in this group too high. Furthermore, of mice that received large doses of estrogen or anterior hypophysial transplants, the proportion of those in PP and CP to those in RP would have been still larger than in the controls if the age level had been the same in both cases, but actually the mortality among mice given large doses of estrogen was much greater in the first age period than among controls. If one keeps in mind these

reservations as to the statistical treatment of our findings, it is possible to draw certain additional conclusions from the data presented in tables 5 and 6.

From the data in table 5 we may conclude that the proportion of mice which pass from the phase of preparatory growth to that of cancerous growth increases in the direction from the low tumor rate strains by way of the medium tumor rate strains to the high tumor rate strains, and that this holds good in the control mice, exposed to the action of ordinary ovarian hormones, as well as in mice which received additional doses of estrogen. The progress from the stage of

TABLE 5.—*Ratio of Number of Mice in CP to Number of Mice in PP*

Strains	Control Mice	Control Mice (With Inclusion of All Tumor Mice)	Mice Given Large Doses of Estrogen
C57 + CBA	2 : 6 = 0.33	(13 : 6 = 2.1)	0 : 63 = 0
Old Buffalo + New Buffalo	9 : 12 = 0.75	(66 : 12 = 5.5)	8 : 19 = 0.42
A	6 : 8 = 0.75	(163 : 8 = 20.4)	22 : 22 = 1
D + C3H	33 : 11 = 3	(503 : 11 = 45.5)	41 : 38 = 1.1

TABLE 6.—*Relation Between Age and Grade in Controls and in Estrogen-Treated Mice*

Strains	Average Age in PP in Controls / PP with Large Doses of Estrogen	Average Age in CP in Controls / CP with Large Doses of Estrogen	(With Inclusion of All Tumors)	Grades in PP in Controls / PP with Large Doses of Estrogen
C57 + CBA	15.6 : 8.65 = 1.8	16 : 0		1.54 : 1.81 = 0.85
Old Buffalo + New Buffalo	16.5 : 7.3 = 2.3	19.3 : 13.5 = 1.4	(17.1 : 13.5 = 1.3)	1.64 : 2.1 = 0.78
A	13.6 : 9.9 = 1.4	13.25 : 9.6 = 1.38	(11.4 : 9.6 = 1.2)	1.84 : 1.98 = 0.93
D + C3H	12.9 : 6 = 2.15	13.6 : 9.1 = 1.5	(10.9 : 9.1 = 1.2)	1.8 : 1.92 = 0.94

preparatory growth to that of cancerous growth takes place, therefore, the more readily the higher the tumor rate of the mice. However, the differences between the various strains were less marked in the animals which received large doses of estrogen than in the control mice. Large doses of estrogen act as a stimulus which tends to a certain extent to equalize the reactions in high and low tumor rate strains.

The figures in table 6 indicate that in a general way in control mice the average age in PP decreases in the direction from the low tumor rate strains to the high tumor rate strains. It furthermore shows that in all the strains the average age in PP is lower in mice which have received large doses of estrogen than in the control mice. In the former, the average age in PP likewise decreases in the direction from the low

tumor rate strain to the high tumor rate strain, except that the figure for strain A is too high. We may then conclude that in general mice progress from the growth-resistant state to the preparatory growth period of the mammary gland the more readily the greater the number of spontaneous mammary gland tumors in these strains. Administration of large doses of estrogen accelerates this transition.

In addition, it may be concluded that in control mice the intensity of this growth as expressed in the average grades is greater in the high tumor rate strains than in the low tumor rate strains and that it is greater in mice which received large doses of estrogen than in the control mice. However, the proportions between the average ages in PP in controls and in mice given large doses of estrogen are variable. The proportions between the average intensities of growth in PP in control mice and in mice given large doses of estrogen are somewhat greater in high tumor rate strains, which fact indicates that the effect of the injections of estrogen may perhaps be less in these mice than in low tumor rate strains, although it is not certain that the observed differences are significant.

It follows from these observations that the growth processes preceding formation of mammary gland carcinoma in mice vary in different strains approximately in accordance with the tendency for tumors to develop in this organ in these strains. With this conclusion agree the observations of Gardner, Diddle, Smith, Strong and Allen,[5] who found in the dissected mammary glands of mice belonging to strains differing in their tumor incidence, which had been given injections of estrogen, scattered small nodules inserted in the duct system. These nodules were least numerous in the strains which had the lowest incidence of mammary gland carcinoma. In general, they increased in frequency with increasing age of the mice. Microscopically, they consisted of proliferating and secreting mammary gland tissue, but they were not, as a rule, remnants of a pregnancy or a lactating gland. These authors believe that mammary gland carcinoma of mice probably has its origin in these nodules. Bonser [6] compared the effect of large doses of estrone (theelin) on the mammary gland in CBA and A mice. In the cancer-susceptible strain there was more localized, but more active, proliferation of acini than in the cancer-resistant strain. Acinous growth was not noticeable in the cancer-susceptible strain until after about forty weeks of treatment.

We have found gradations in the reactions of the mammary gland to growth stimuli in the various strains of mice which we have examined; these reactions are evaluated on the basis of the microscopic examination of great parts of this organ in each animal. It may then be concluded that in a general way the strains with a strong hereditary tendency toward mammary gland carcinoma (strains D and C_3H) respond to

growth stimuli more rapidly with a transition from RP to PP than do strains with a low hereditary tendency. This applies to mice in which the growth stimuli are supplied by the normal ovarian hormones, leading to formation of "spontaneous" cancer, as well as to those in which the stimuli are furnished by experimentally administered estrogen or by transplanted hypophysial glands. The mice with a strong hereditary tendency toward the development of mammary gland cancer likewise pass more rapidly through PP and reach thus the precancerous and cancerous stage earlier than the others. Moreover, the reason that a strain with a very low tendency toward the development of mammary gland carcinoma does not, as a rule, reach the cancer stage may not depend on a special difficulty encountered in entering the cancerous phase after having passed through the phase of preparatory growth but on the insufficient progress that such mice make in the latter phase. Furthermore, the administration of large doses of estrogen very clearly accelerates the transition from RP to PP and from PP to CP. It appears, then, that the differences in the hereditary tendency toward the development of mammary gland carcinoma in various strains of mice depend on the readiness with which the mice belonging to these strains respond to the action of continued or often-repeated hormonal stimulation of growth with growth processes of a progressive kind. The latter must be distinguished from the ordinary rhythmic growth processes characteristic of the sexual cycle, which may eventuate in those progressive growth processes leading to the production of mammary gland carcinoma.

SUMMARY

A statistical study of 756 mice, in which a microscopic examination of sex organs and certain other organs was made, confirmed the conclusion that the various strains of mice that reach the stage of cancerous growth of the mammary gland pass first from the phase of resistance to growth to a phase of preparatory growth and that the difference in the readiness with which the cancerous phase is reached depends not only on the readiness with which mice pass from the stage of preparatory growth by way of a precancerous stage to the fully developed carcinoma but also on the readiness with which mice pass from the first phase, that of resistance to growth, to the preparatory growth phase and on the rapidity with which they pass through the stage of preparatory growth. The readiness with which the mammary glands of various strains respond with the progressive growth that is characteristic of the preparatory growth phase to long-continued stimulation by endogenous or exogenous estrogenic substances is at least one of the factors determining the difference in the hereditary tendency of various strains of mice toward the development of mammary gland carcinoma.

RELATION OF THE "ANITSCHKOW MYOCYTE" TO RHEUMATIC INFLAMMATION

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The "Anitschkow myocyte" is a cell with a peculiar nuclear structure found normally in the heart and heart valves. It has never been seen in other organs. It was called a myocyte by Anitschkow [1] because he believed the cell had its origin in the muscle fibers of the heart. Ehrlich and Lapan,[2] on the other hand, were convinced that this cell arose from the interstitial tissues of the heart and preferred to call it a myocardial reticulocyte. Since the term "reticulocyte" is applied to a red blood cell, Downey [3] has suggested that "histiocyte" would be a better name for this morphologically specialized cell.

Ehrlich and Lapan described the nucleus as being elliptic and vacuolated except for a serrated bar of chromatin material in the center of the nucleus with fine fibrillar structures extending at right angles from the central bar toward and in many cases to the nuclear membrane. In cross section the nucleus is round or nearly so with a dark chromatin mass in the center. Radiating chromatin fibrillae are seen as in the longitudinal sections. In normal hearts very little cytoplasm is seen in the cell, but when inflammation is present, the cytoplasm enlarges and takes on a darker stain. Ehrlich and Lapan found this Anitschkow nucleus in normal embryonal and in adult hearts in many kinds of vertebrate animals. They concluded that the Anitschkow myocyte is a normal constituent of the human heart during the embryonal and post-embryonal stages of development and that the cell is a part of the supporting tissue of the heart and belongs to the fixed elements of the reticuloendothelial system. It plays a definite role in inflammatory and other defense reactions.

Jacki [4] and Wätjen,[5] both of whom have made extensive studies on rheumatic inflammation, noted a resemblance of the nucleus in the Anitschkow myocyte to the nuclei in some of the cells in Aschoff nodules.

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1. Anitschkow, N. H.: Beitr. z. path. Anat. u. z. allg. Path. **55**:373, 1913.
2. Ehrlich, J. C., and Lapan, B.: Arch. Path. **28**:361, 1939.
3. Downey, H.: Personal communication to the author.
4. Jacki, E.: Frankfurt. Ztschr. f. Path. **22**:82, 1919.
5. Wätjen: Verhandl. d. deutsch. path. Gesellsch. **18**:223, 1921.

Wenezianowa-Grusdkowa [6] found that this cell is capable of storing dyes and concluded that the cell has phagocytic properties.

Proliferative and reactive changes in this cell in the heart were observed by Ehrlich and Lapan in a number of pathologic states, such as sepsis, meningococcic bacteremia, scarlet fever, periarteritis nodosa and subacute bacterial endocarditis. They observed, as Jacki and Wätjen had, that the nucleus in the Anitschkow cell resembled nuclei in some cells in Aschoff nodules.

The foregoing observations stimulated my interest in the frequency of this type of cell in rheumatic inflammation in the myocardium, heart valves and subcutaneous nodules in patients dying in attacks of acute or recurrent rheumatic endocarditis. An attempt was made to determine whether the presence of this characteristic cell might be a basis for the belief that there is a typical Aschoff nodule or that the Aschoff nodule is a specific type of inflammation.

MATERIAL

Thirty-six cases of acute or recurrent rheumatic endocarditis in which Aschoff nodules were found in the heart were examined for the presence and relative number of the Anitschkow cells in the nodules. Several nodules were examined in each case. Subcutaneous nodules in cases of acute rheumatic fever and chronic arthritis and streptococcic experimental myocardial and subcutaneous nodules were also studied.

The degrees of involvement were expressed by ++++, +++, ++ and +. The degree ++++ represented the nodules in which most of the cells were the Anitschkow type. The degree + was assigned to the nodules that showed only an occasional Anitschkow cell or about the number seen in the normal heart. Grades +++ and ++ were intermediate degrees.

RESULTS

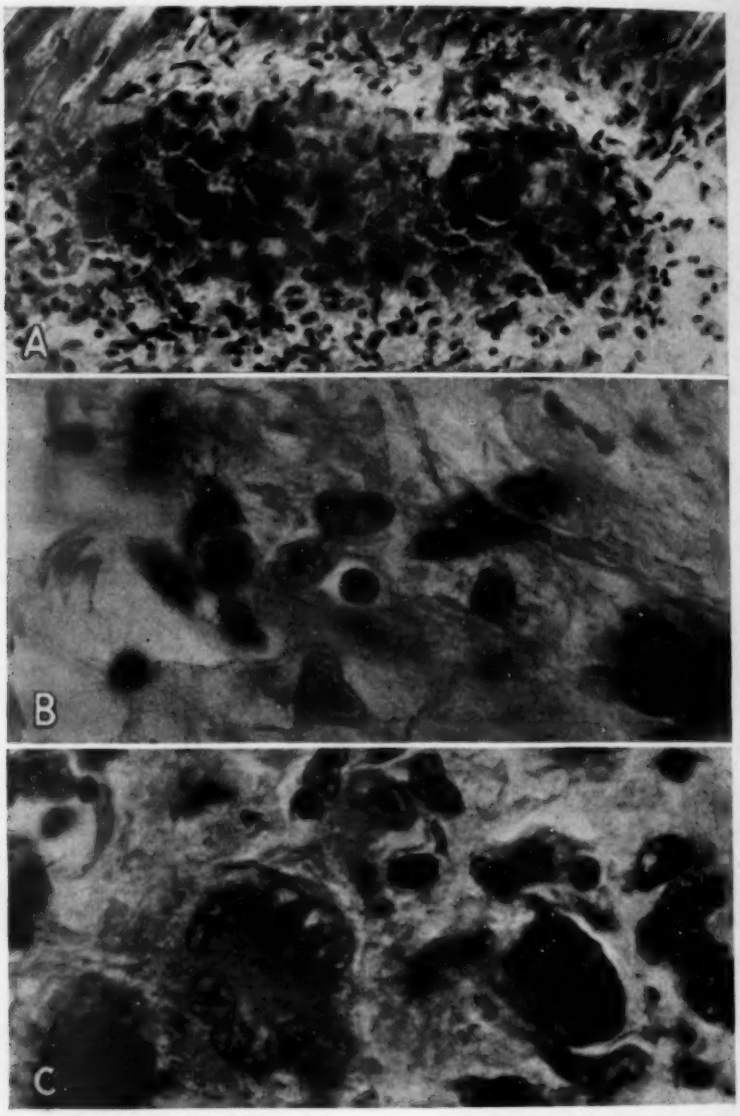
The relative degree of Anitschkow cellular involvement was determined in 36 cases of rheumatic endocarditis with Aschoff nodules; in 6 the degree was + + + +; in 14, + + +; in 11, + +, and in 5, +. Nodules without Anitschkow cells were not found.

The figure shows two Aschoff nodules and nuclei scattered in and about the nodules and in giant cells of the nodules. It was unusual to find giant cells containing other kinds of nuclei, and giant cells containing other nuclei with the Anitschkow nuclei were never encountered.

The Anitschkow cells were found in the hearts of rabbits in nodules produced by injecting streptococci in our own material and in an experimental cardiac nodule reported by Jackson.[7] No Anitschkow cells were

6. Wenezianowa-Grusdkowa, M. S.: Frankfurt. Ztschr. f. Path. **37**:538, 1929.

found in subcutaneous nodules from patients having acute rheumatic fever or chronic arthritis or in subcutaneous nodules produced experimentally in rabbits.



A, Aschoff nodules in which the Anitschkow cells were observed; *B*, the Anitschkow nuclei in an Aschoff nodule; *C*, the Anitschkow nuclei in giant cells in an Aschoff nodule.

7. Jackson, L.: J. Infect. Dis. **11**:243, 1912.

COMMENT

The "Anitschkow myocyte" probably should be considered a cardiac histiocyte, since it is now generally agreed that it develops from cardiac interstitial tissues and not from the myocardial muscular fibers. It appears to be a cell which is restricted in its location to the heart and heart valves. It is normally seen in the interstitial tissues of the heart and shows marked change and proliferation in rheumatic inflammation, but other histiocytes are also present.

The special histiocyte is often the chief cell found in the Aschoff nodule. It is increased and changed to a greater extent in rheumatic inflammation than in syphilitic inflammation (gumma) of the heart. The presence of the Anitschkow cell in the Aschoff nodule is due to the fact that this cell is present in the heart and not to the fact that the cell responds specifically in rheumatic inflammation. This conclusion is brought out by the failure of the cell to appear in subcutaneous nodules in acute rheumatic fever. The presence of the Anitschkow cell in Aschoff nodules may help to explain the meaning of typical Aschoff nodules.

SUMMARY

The "Anitschkow myocyte," the myocardial reticulocyte (Ehrlich and Lapan) or the cardiac histiocyte, is normally found in the heart and heart valves.

It responds in inflammation by an increase in cytoplasm and is often the chief cell to respond in rheumatic inflammation and in experimental inflammation in the heart.

It is not a characteristic cellular response in rheumatic inflammation, for it is not found in rheumatic subcutaneous nodules.

KAPOSI'S DISEASE

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Kaposi's so-called "multiple idiopathic hemorrhagic and pigmented sarcoma" manifests itself most often in the skin, although it may present changes in the deeper parts, notably in the gastrointestinal tract, which are comparable in many respects with those in the skin. The disease has naturally attracted the attention of dermatologists, but among other clinicians it is not commonly recognized, and apparently is not even very widely known. It has been variously interpreted by those who specialize incidentally in the histopathology of the diseases and disorders of the skin as a round cell sarcoma (Kaposi), angiosarcoma, acrosarcoid, granuloma, hemangioendothelioma, perithelioma, myoneurovascular dysgenesis, endoperithelioma, systemic angiomatosis, pseudosarcoma, hamartoma, angioreticuloendothelioma, the proliferation of organizing connective tissue with vascular dilatation, and so on. The disease seems to have received scant attention from pathologists, the results of investigation by necropsy having been recorded in only a few instances. This appears to be due in part at least to the fact that the disease occurs with greatest frequency among those who are racially or otherwise opposed to the rite of necropsy.

In the present paper it is shown, I believe, that the unit of growth in Kaposi's disease is the fibroblast. The behavior of this cell determines the course of the disease in each of its several phases, excepting, of course, such incidental features as hemorrhage and pigmentation. Thus the fibroblast may act in such fashion as to bring about spontaneous replacement of individual nodules, or it may maintain a low capacity for growth over a long period of years, or it may suddenly take on highly malignant qualities and terminate life by the process of metastasis. A tumor of the latter description is recorded here (case 8). It apparently originated in the stomach about ten years after primary involvement of the skin.

CLINICAL FEATURES

Kaposi's disease is remarkable, among other things, for its long duration. Its average span is estimated to be between eight and ten years, but in a number of instances it has been known to persist for

From the Laboratories of Pathology, Bellevue Hospital.

periods of from twenty-five to forty-one years. On the other hand, in one of the Bellevue Hospital cases recorded in this paper the disease became widely disseminated in the course of one month and four days. The disease is not common nor is it extremely rare. Nothing is known of its immediate cause, but there are several factors which appear to be contributory. In by far the larger number of cases it is encountered in Jews and Italians over 40 years of age, and in a large percentage of all cases, in individuals between 60 and 90 years of age. It has been described in members of many different nationalities but is rare in full-blooded Negroes [1] and is practically unknown in mulattoes. In England, for reasons which are not apparent, it is scarcely known, only 9 cases having been recorded up to the year 1940. In that part of the literature of Kaposi's disease with which I am acquainted and in my own clinical contacts with it I have not encountered a case among Mongolians. According to these observations, the disease seems to be rare among Anglo-Saxon peoples. It occurs in males in about 91 per cent of cases. With only rare exceptions, it is found in those whose occupations entail manual labor—dishwashers, bakers, fishermen, housewives, blacksmiths, shoemakers and the like. It is restricted in a high percentage of cases to those who live in unfavorable urban conditions and whose diets are probably poorly balanced.

Clinically, the disease is so sharply set apart that once the physician has become familiar with its visible lesions there is no cutaneous disorder with which he is apt to confuse it, although I have known it to be mistaken for Boeck's sarcoid, xeroderma pigmentosum and even acrodermatitis chronica atrophicans. The predominant growths are in the skin; changes in the visible mucous membranes are rare. The lesions occur usually in multiples and not infrequently are present by dozens, sometimes by hundreds, rarely by ones or twos. Even in the earlier stages of their evolution, the nodules in the skin may be recognized by inspection. The growths in the visible mucous membranes seem always to be associated with similar growths in the skin, and were it not for this association, they might easily escape clinical identification. When the disease is well developed, the skin presents an array of reddish or purplish nodules, plaques and plateau-like formations. As a general rule, the smaller nodules are rounded, the plaques present a flat or nodulated surface, and the plateau-like growths are irregularly nodular and undulating. The individual nodules vary in diameter from a few millimeters to 2 or 3 cm.; the plaques and the plateau-like growths may reach enormous proportions, varying from 5 to 20 cm. or more. The nodules do not seem to coalesce; the plaques and plateaus appear to represent the expansion of multiple large and small nodules which are

1. Ellis, F. A.: Arch. Dermat. & Syph. **30**:706, 1934.

closely apposed to one another. The distribution of the changes in the skin is interesting, but its significance is not clear. The growths occur in greatest abundance, in my experience at least, in the skin immediately covering bone and in lesser numbers in the skin over soft tissues. Most often the lesions appear first on the lower extremities, usually on the toes, around the ankle joints and in the lower pretibial regions, but not uncommonly occur first on the fingers, around the wrists and on the lower part of the forearm, sooner or later becoming symmetric in the extremities concerned. They appear in scattered and relatively small numbers in the skin of the abdominal wall and of the calves, the thighs, the buttocks and the arms, and still less often in the skin of the back and chest. The plateau-like formations are apt to attain their greatest size in the immediate vicinity of joints, notably the small joints of the hands or feet. In other words, the cutaneous lesions seem to express a preference for those regions where the skin is less flexible and the blood supply less plentiful and to avoid those parts where the skin is more easily movable and the supply of blood is more abundant.

Another remarkable feature in the clinical display of Kaposi's disease is the frequency with which certain of the smaller nodules lose their reddish or purplish color, take on a yellowish appearance faintly speckled by red, sometimes by light green, and disappear gradually and without assistance, leaving smooth whitish or brownish scars, to be replaced in time by new nodules in the immediate vicinity or at a distance from the old.

As the disease progresses the collagenous tissues in the vicinity of the nodules become thickened, lymph stasis occurs and the skin is thrown into folds so that the extremity presents much the same appearance as that involved by the nonparasitic variety of elephantiasis. Finally, the growths may ulcerate, usually as a result of trauma, sometimes producing superficial excoriations and at other times open lesions of formidable dimensions.

In the wards of Bellevue Hospital in the past twenty-five years I have had occasion to observe 25 patients with Kaposi's disease and to study the histologic changes as revealed at biopsy and, in 1 instance, at necropsy. Eight cases have been assembled for the purpose of this record because each of them presents certain features which appear to me to be worthy of note. The others have been omitted to avoid repetition of clinical and histologic detail.

REPORT OF SEVEN CASES OBSERVED CLINICALLY

CASE 1.—S. C., aged 63, an Italian housewife, was admitted to Columbus Hospital, May 24, 1940, complaining of pain in the right upper quadrant of the abdomen of eight days' duration, vomiting of greenish fluid material and obstinate constipation. At the same time she was jaundiced. Cholecystectomy was done. The

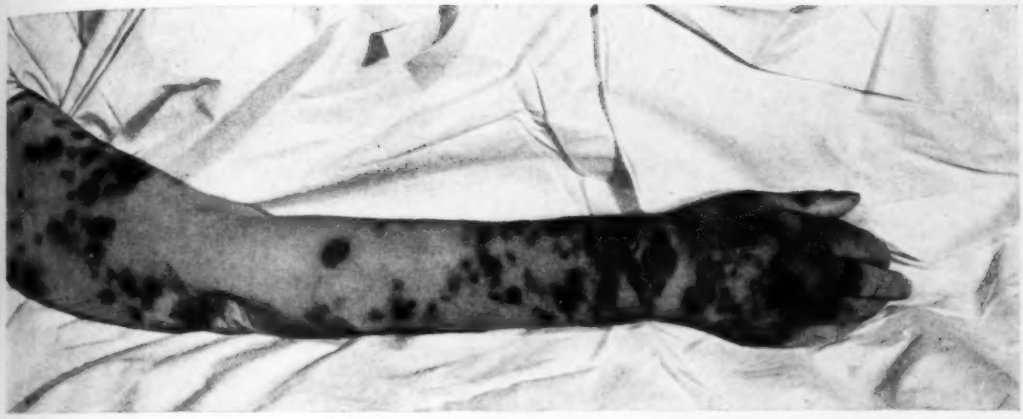


Fig. 1 (case 1).—Color photograph showing the distribution of the lesions in Kaposi's disease in the upper extremity of a jaundiced Italian woman. In this patient both upper extremities were involved to an extraordinary extent and parts of the trunk to a lesser extent in the course of one month and four days. Death resulted from intercurrent disease. (The average duration of Kaposi's disease is from eight to ten years.)

gallbladder was found to be markedly thickened. It was distended by pasty bile, and in it were a number of stones; one was lodged in the cystic duct. The patient improved slightly after operation and was discharged on August 30. She was at that time deeply jaundiced. On September 20 she was admitted to Bellevue Hospital and died October 3. In addition to marked jaundice, dyspnea, cyanosis and edema of the lower extremities, she presented multiple dark reddish blue or purplish nodules in various parts of the body. She stated that these nodules first made their appearance in the region of the head, just before she was discharged from Columbus Hospital. However, a copy of the history obtained from Columbus Hospital made no mention of them. Dr. Andrea Saccone, pathologist at Columbus Hospital, was good enough to investigate this phase of the patient's history and was unable to confirm it. At the time of the patient's admission to Bellevue Hospital she presented innumerable reddish or bluish nodules in both upper extremities, the nodules varying from 0.5 to 5 or 6 cm. in diameter (fig. 1). The larger ones were arranged in groups in the region of both wrists and the fingers. Similar but somewhat smaller nodular formations were scattered in some profusion through the forearms and the lower part of the arms. In several instances it was noted that the nodules were only slightly elevated or almost flush with the surface of the skin and were rather firm in consistence. These were probably nodules in the process of regression. Between the larger nodules on the hands and forearms were numbers of bright reddish macular areas representing, most likely, nodules in the process of early formation. Scattered through the skin of the forehead, scalp and face were a half dozen nodules, measuring from 0.5 to 1.5 cm. in diameter. There were a few nodules in the skin of the chest, back and abdomen, varying from 0.5 to 2 cm. in diameter, while in the skin of the thighs and legs there were occasional nodules, measuring 0.5 to 1 cm. in diameter.

The duration of Kaposi's disease in this instance was apparently one month and four days. Permission for necropsy was not obtainable.

Microscopic sections of the excised gallbladder were examined by Dr. Saccone and myself. The changes were those of an extensive chronic productive cholecystitis.

Microscopic examination of a nodule removed from the skin during life showed a fibroblastic new growth which was richly infiltrated by red cells. The nodule was almost completely circumscribed by a thin layer of rather loosely arranged collagenous fibrils and fibroblasts, some of the latter lying parallel with one another. In and just beneath this layer were numbers of dilated endothelial channels containing red cells. Some of these channels showed points of rupture with immense numbers of red cells lying in the immediate vicinity. The substance of the new growth was composed of fibroblasts, cut both longitudinally and crosswise. The fibroblasts were loosely arranged, and among them were red cells occurring by twos and threes or in the form of small pool-like collections. No pigment was apparent, biliary or otherwise.

Comment.—This case in which the patient was a female is also remarkable because of the extreme rapidity with which the disease involved the greater part of both upper extremities and appeared as scattered nodules in other parts of the body, including the face and scalp, death occurring from intercurrent causes one month and four days after the appearance of the first nodules in the skin. Although I have never seen or heard of Kaposi's disease in a jaundiced subject, perhaps it would not be too

presumptuous to assume that hemorrhage into the nodules might be encouraged or even initiated by bile salts. Further than this jaundice should be regarded as an incident of no apparent importance.

CASE 2.—A. R., aged 86, an Italian housewife, was admitted to Bellevue Hospital, May 22, 1935, because of senile dementia. Examination revealed nothing of importance in the present connection except for the presence at the tip of the fourth toe on the left side of a firm, nontender, bluish red, superficially ulcerated area, 6 mm. in diameter. There were no similar formations in any other part of the body. The patient's mental condition was such that it was impossible to obtain from her any history as to the length of time that this growth had been present.

Microscopic examination showed superficial infection and ulceration, beneath which there was a spindle cell new growth, measuring 5 mm. in its longest diameter. Between the latter and the ulcerated surface were a half-dozen or more endothelial channels, which were distended by red cells. The spindle cells for the greater part lay parallel with one another and were arranged in interlacing bundles. Between them were vast numbers of extravasated red cells, most of which stained poorly and were evidently beginning to disintegrate. In the spindle cells mitotic figures were numerous. No pigment was visible.

Comment.—Except for the fact that the lesion was solitary and that it occurred in a woman, this case presented nothing worthy of further note. A second case of solitary growths is recorded in this paper (case 7). In a third case at Bellevue Hospital the patient, an Italian tailor aged 62, presented two growths only. Both were situated on the inner aspect of the left ankle and measured about 0.5 cm. in diameter and lay within 0.5 cm. of one another.

CASE 3.—M. C., aged 56, an American Negro, a laborer, was admitted to Bellevue Hospital Nov. 24, 1939 and died Feb. 13, 1940. On May 17, 1938 he was admitted to the outpatient clinic of Harlem Hospital, complaining of a painful indurated area at the tip of the little finger on the right hand. There was no history of injury to the part. Eight months later he was readmitted to the outpatient clinic. At that time the indurated area was nodulated, and a small piece of tissue was removed for biopsy. On the occasion of his first admission to Harlem Hospital the patient gave no history of a chancre but stated that several years before he had had a "papillar" rash. At Harlem Hospital the patient's Kahn reaction was 4 plus, and he received 17 injections of a bismuth compound between June and November 1939. On admission to Bellevue Hospital he complained of swelling of both upper and lower extremities of ten months' duration together with a "rash" over both arms and legs. Physical examination revealed a disorder of the type of dermatitis exfoliativa involving all extremities. In the skin covering the left hypothenar eminence were several bluish red nodules. In the skin of the right hand there was a collection of similar nodules at the tip of the little finger, as well as a large nodulated plaque in the region of the thenar eminence and one in the palm of the hand, a series of nodules along the hypothenar eminence and a small plaque in the skin covering the right wrist joint just above the palm of the hand (fig. 2). In the skin covering both ankle joints there were twenty or more similar nodules, each measuring about 0.5 cm. in diameter.

Microscopic examination of tissue removed at Harlem Hospital showed immediately under the epidermis a layer of hyalinized connective tissue in which were innumerable endothelium-lined blood sinuses partially or completely filled, or even markedly distended, by red cells. Abutting directly on this layer was a richly fibroblastic new growth, scattered through which were innumerable hemorrhagic extravasations and large numbers of golden brown pigment deposits. Deep in the derma just beyond the confines of the new growth were a few sweat glands, and in the connective tissues around them were rich deposits of the same sort of pigment. Microscopic examination of tissue removed at Bellevue Hospital showed essentially the same changes except for almost complete absence of pigment.

Comment.—This case is of interest because of the rarity of Kaposi's disease in full-blooded Negroes. Pardo-Castello in 1931 and Ellis [1] in 1934 each reported a similar case. The one recorded here is believed to be the third in the literature on Kaposi's disease. Likewise, in the

Fig. 2 (case 3).—The right hand of a full-blooded American Negro, showing lesions on the anterior surface of the wrist and on the hypothenar and thenar eminences, the palm and the tip of the little finger.

case here recorded the patient presented presumptive evidence of syphilis. There have been few attempts to establish syphilis as a factor in any of the manifestations of Kaposi's disease and this record is not intended as an addition.

Case 4.—J. F., an Italian aged 78, unemployed, was admitted to the department of radiation therapy of Bellevue Hospital, May 12, 1932, complaining of itching over both legs of about a year's duration. The itching commenced in the region of both ankles and was intense and constant. After prolonged scratching the patient noticed several small reddish nodules in the corresponding areas. Shortly thereafter other and similar nodules appeared in different parts of both lower extremities. Three months before he came under observation, other reddish nodules appeared in the region of the left mastoid process and about the scrotum, and more recently the left hand became similarly involved. Itching in all of the affected

parts was persistent and at times was almost unbearable. Physical examination revealed pronounced nonpitting edema of both lower extremities together with a diffuse purplish discoloration of the skin, which was attributed to cyanosis. The legs and the lowermost parts of both thighs were richly spotted by reddish nodules, which varied in diameter up to 5 cm. The nodules throughout were discrete and firm and blanched slightly on pressure. They were tender and appeared to be fixed to the overlying structures but were freely movable against the deeper tissues. In the left thigh there was a bright reddish, slightly elevated plateau-like formation, which measured 5 cm. in diameter, and the region of the left mastoid was similarly occupied by a large, flattened, diffusely reddish growth. While the patient was under observation, new nodules developed in the scalp and neck, in the region of both knees and over both aspects of both feet, and the nodules in the legs increased greatly in number, some of them becoming ulcerated. The patient stated that on numerous occasions he had noticed that some of the smaller nodules disappeared spontaneously. Finally the skin of the legs and ankles became brawny, indurated, deep brownish and thrown into coarse folds, so that the extremities resembled those involved in elephantiasis.

Microscopic examination of two small pieces of cutaneous nodules that were removed at different times showed bundles of spindle cells, some of which were arranged compactly and others of which were separated for variable distances by infiltrating red blood cells. In places the spindle cells were cut transversely and resembled collections of large round cells; in other places they appeared as small holes containing red cells. Scattered through the growths were numerous endothelial channels distended by red cells. Several hemorrhagic extravasations were present in both nodules. Deep in the substance of one of the growths was a small solitary rounded nodule surrounded by a thick band of collagenous fibrils enclosing spindle cells cut both longitudinally and transversely, several slightly distended endothelial vessels and one or two small hemorrhages. No pigment was observed in either section.

Case 5.—O. G., aged 32, a Jewish man, was seen in the department of radiation therapy of Bellevue Hospital May 19, 1932. He complained of several "pimples" on both legs. One of the pimples was ulcerated. At this time there was no complaint of itching, but as similar lesions appeared in other parts and became more numerous, itching became intense and persistent. Physical examination showed numbers of reddish or purplish nodules in the skin of both legs from the ankles to the knees. The individual nodules measured from 0.5 to 1 cm. in diameter. There was a solitary but similar nodule in the skin of the left forearm. Both lower extremities were diffusely bronzed and edematous. The patient was under observation for a period of about eight years, during which time numbers of additional lesions appeared on the legs, the right hand, the left wrist, both feet and thighs and the right forearm, aggregating a total of many dozens. The patient himself noted that from time to time the nodules in different situations disappeared spontaneously, sometimes leaving brownish areas in their stead. A segment of one of the nodules in the leg was removed for biopsy.

Examined in March 1941, the patient showed remarkable improvement. At this time there was a solitary slightly reddish nodule just below the ankle joint on the right side. The nodule measured about 1 cm. in diameter. The skin of the corresponding foot was moderately thickened and diffusely bronzed. The left leg and foot were apparently normal. Otherwise there was nothing worthy of note except that the patient stated that for a year past he had been tormented by

generalized itching, especially at night, when he was in bed. The patient attributed his improvement to prompt radiation treatment whenever a new nodule presented itself.

Microscopic examination showed a segment of a nodule surmounted by moderately hyperplastic epidermis. Immediately beneath was a greatly thickened band composed of collagenous connective tissue bundles, some of them hyalinized. Strewn among the collagenous bundles were variable numbers of poorly chromatic plump spindle-shaped cells arranged irregularly in groups; others lay parallel with one another and were compact. In places the spindle cells were separated to form oval or elongated cavernous spaces, which were filled with red cells. In other places the bundles supported moderately distended endothelial channels. The substance of the growth proper was composed of rather long spindle cells, some of which were arranged thickly in parallel formation and others irregularly and loosely. In a few instances minute granules of golden brown pigment were seen.

Comment.—In this, as in the preceding case, intense and persistent itching was complained of by the patient. This symptom is unusual in Kaposi's disease. The scratching incidental to itching has been invoked to explain a traumatic conception for the origin of the growths in the skin, but this view seems scarcely worthy of serious consideration. In both of these cases, likewise, spontaneous disappearance of small nodules was noted by the patients themselves.

Case 6.—G. U., an Italian shoelace maker, was admitted to the eye service of Bellevue Hospital, May 20, 1935, complaining that his left eye had been troubling him for three weeks past. Examination showed an oval new growth located in the conjunctiva immediately adjacent to the upper and outer edge of the left cornea. The growth was movable and nonpigmented, and measured about 5 mm. in its longest diameter. The vessels around it were injected in spider-like fashion. The growth was removed but recurred, and the patient was readmitted to Bellevue Hospital Aug. 3, 1936, at which time he presented a growth at the site of the former removal and one immediately beneath it, the two being connected by a delicate bridge of apparently the same sort of tissue. The recurrent and the new growth each measured about 5 mm. in longest diameter, and both were bright reddish and nonpigmented. There was a similar growth in the skin of the temporal side of the left lower eyelid. In addition, examination showed similar growths in other parts of the body, the individual nodules varying from a few millimeters to 2 or 3 cm. in diameter. The patient stated that the latter growths had been present for four years. Growths occurred with noticeable frequency in the skin of the dorsal and palmar surfaces of the left hand and on both surfaces of the left wrist. The corresponding areas in the right hand and wrist were likewise involved but not to nearly the same extent. In the region of the left knee there were about twenty lesions of like character and two in the region of the right knee. The skin of the posterior axillary folds presented a half dozen similar lesions on each side. The nodules were firm, painless, reddish or reddish blue and nonpigmented. The left hand and the right foot showed marked pitting edema, and the dorsal surfaces of both feet were diffusely brownish.

Microscopic examination of sections from the nodules in the conjunctiva showed partial or complete encapsulation and trabeculation by collagenous connective tissue. In this manner the growths were irregularly divided into islands of spindle cells, most of the cells lying parallel with one another; others were cut crosswise (fig. 3). In the spindle cells mitotic figures were fairly abundant (fig. 4). Red blood cells

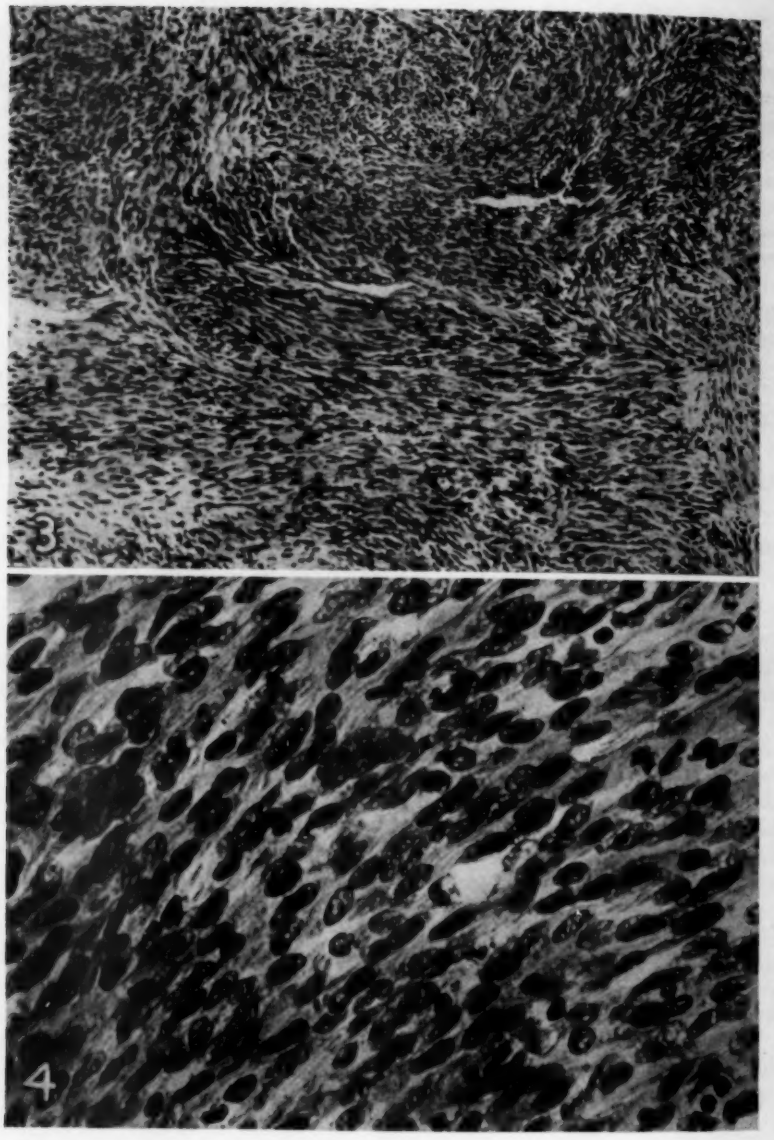


Fig. 3 (case 6).—Photomicrograph of a section removed from a nodule in the conjunctiva, showing collections of spindle cells cut longitudinally and crosswise. Hematoxylin and eosin; paraffin section; × 130.

Fig. 4 (case 6).—Photomicrograph of spindle cells with mitotic figures and abundant intercellular collagen. Same section as figure 3; hematoxylin and eosin; paraffin section; × 300.

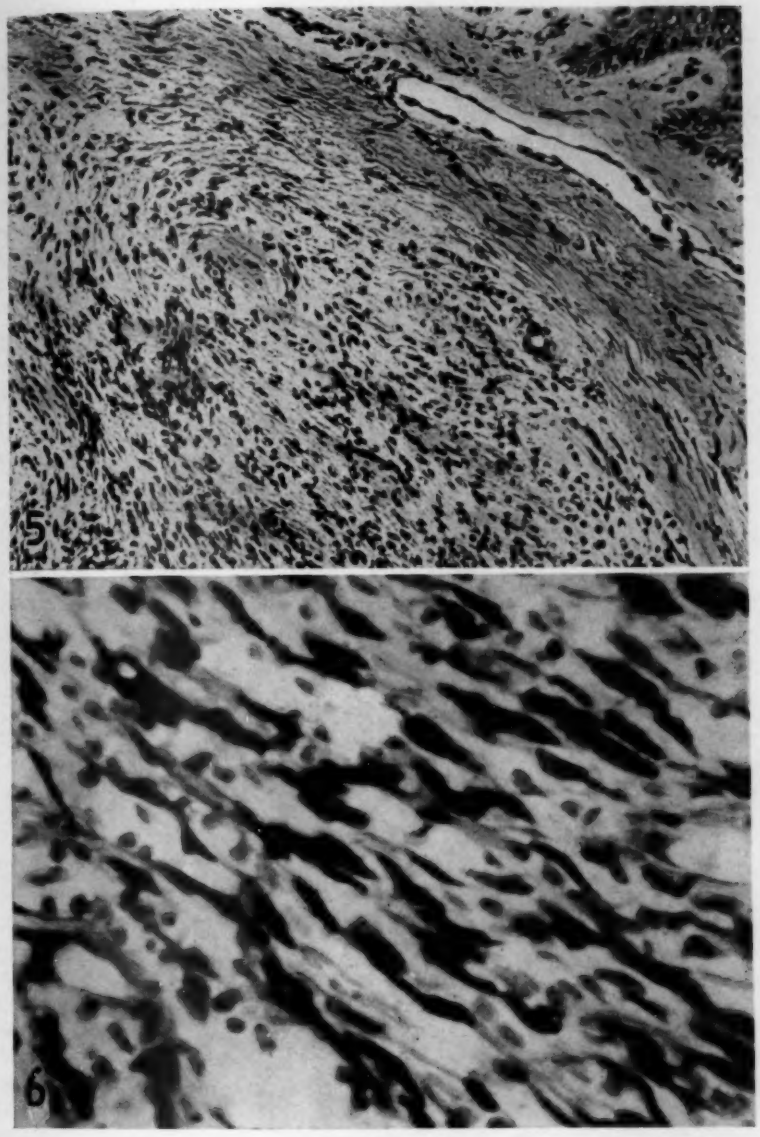


Fig. 5 (case 6).—Photomicrograph of a section from skin showing peripheral dilated and elongated endothelial channels and fibroblastic growth originating apparently in the upper derma. Hematoxylin and eosin; paraffin section; × 112.

Fig. 6 (case 6).—Photomicrograph showing faded red blood cells lying between degenerate and disintegrating fibroblasts. Hematoxylin and eosin: paraffin section: × 660.

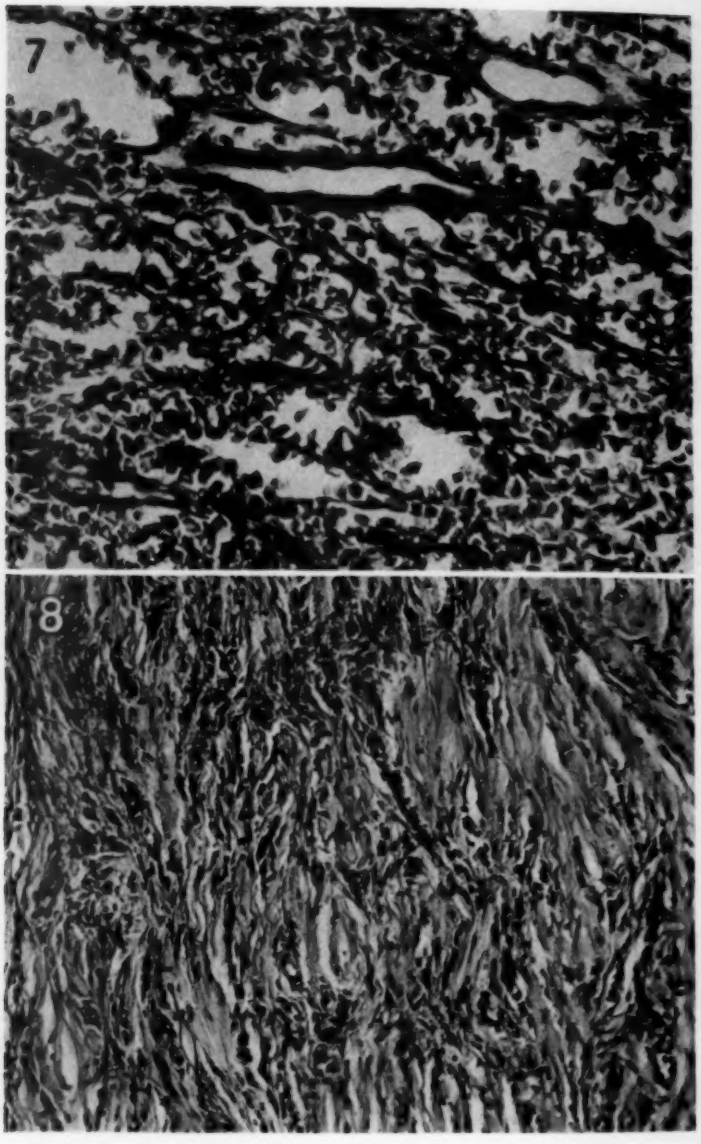


Fig. 7 (case 6).—Photomicrograph showing distorted red blood cells in "sinusoids" formed by fairly well preserved fibroblasts. Hematoxylin and eosin; paraffin section; × 300.

Fig. 8 (case 8).—Photomicrograph of cutaneous nodule showing almost complete replacement by hyalinized collagenous bundles. The darker areas represent collections of hemosiderin. Hematoxylin and eosin; paraffin section; × 190.

were scattered in some profusion throughout most of the sections, all of them lying between the spindle cells. In the growths, either in the connective tissue trabeculae or among the spindle cells, were endothelial capillaries containing variable numbers of red cells. Minute quantities of yellowish brown pigment were to be seen lying close to or in the coarser collagenous trabeculae.

Microscopic examination of sections from the skin showed a multilobulated spindle cell growth lying in the derma. Between the epidermis and the growth was a fairly thick band of collagenous fibrils in which were many loosely set spindle cells. Some of the uppermost of the collagenous fibrils were hyalinized. Lying in this layer were endothelial channels, partly or completely filled by red cells, and several widely dilated lymph spaces. The connective tissue bands at the periphery penetrated the substance of the growth in such fashion as to divide it into several small lobules. The spindle cells, which made up the greater part of the lobules, were arranged compactly and parallel with one another. At the periphery of the nodules, just under the epidermis, the less well nucleated and presumably younger fibroblasts were directly traceable downward between the better nucleated and presumably older fibroblasts into the structure of the growth proper (fig. 5). Between the fibroblasts at frequent intervals were small collections of red cells. In other places the spindle cells were widely separated, and served to form cavernous sinuses, which were distended by red cells (figs. 6 and 7). No pigment was observed.

Comment.—Conjunctival nodules are not unknown in Kaposi's disease and are usually associated with histologically similar growths in other accessible mucous membranes, such as those of the mouth, pharynx and esophagus, and always, it appears, with corresponding changes in the skin. The conjunctival lesion in this case is believed to be the only one thus far recorded in the literature on Kaposi's disease in the English language and the only recurrent conjunctival growth so far described. Although the cutaneous lesions in Kaposi's disease tend to become symmetric, the conjunctival nodules thus far recorded have been unilateral.

Case 7.—A. U., a Jewish man aged 68, a baker, was admitted to the outpatient department of Bellevue Hospital, April 30, 1936, complaining of vague gastrointestinal symptoms of several years' duration and of a small itching growth on the auricle of the right ear. He stated that he had had a similar growth removed from the same spot a year previously. The growth on the ear was about 3 mm. in diameter and reddish. There were no similar lesions in any other part of the body. The growth was removed for biopsy.

Microscopic examination showed a small richly cellular fibroblastic growth which was surmounted by a markedly thickened epidermis and surrounded by a broad band of collagenous connective tissue. In the connective tissue were numerous endothelial capillaries partially or completely filled by red cells. The fibroblasts at the periphery merged somewhat gradually into the more loosely arranged fibroblasts which made up the center of the growth. In the body of the growth the spindle cells ran parallel with one another; in them mitotic figures were numerous, and intercellular collagen was abundant. Distributed through the central growth were moderate numbers of endothelial capillaries containing red cells and small

numbers of hemorrhagic extravasations. A few small hemorrhagic extravasations were also found in the connective tissue surrounding the growth. No pigment was observed.

Comment.—This case is interesting because of the recurrent unilateral lesions in the auricle of the right ear without corresponding changes in any other part of the body.

REPORT OF A CASE WITH NECROPSY

In that part of the literature of Kaposi's disease available to me I have been able to find reports of only 9 necropsies which had been carried out with due regard for anatomic and microscopic descriptions. Perhaps the most striking single feature from the point of view of pathologic anatomy is the uniformity with which lesions corresponding to those in the skin occur in the submucosa of the gastrointestinal tract, including the esophagus, the stomach and the large and small intestines. However, the combined necropsy observations show that similar lesions may occur in practically every organ in the body—among others, in the trachea, bronchi and lungs, in the heart, spleen, liver, kidneys and adrenal bodies, in the walls of the urinary bladder, in the testicles, in the peritoneum, pleura, and dura mater and in the bones. Phillipson,[2] Dalla Favera [3] and Hansson [4] each have reported a necropsy showing multiple lesions in the skin without visceral involvement. The opposite condition has not been demonstrated, as far as I myself am convinced, namely, primary visceral Kaposi's disease without corresponding changes in the skin.[5]

The nodules in the submocosa of the gastrointestinal tract vary in number from a few to seventy-five or more. They are described as well demarcated, rounded, conical or mushroom-shaped, varying in size from 1 to 4 cm. Some of them present a crater-like depression at the top and are superficially ulcerated; in others the covering epithelium appears to be intact. Seen through the epithelial covering and on cross section, they are whitish or flesh colored or marbled by red and white

2. Phillipson, L.: Virchows Arch. f. path. Anat. **167**:58, 1902.

3. Dalla Favera, G. B.: Arch. f. Dermat. u. Syph. **109**:387, 1911.

4. Hansson, C. J.: Acta radiol. **21**:457, 1940.

5. Dr. G. Louis Weller Jr., of Washington, D. C., in a paper entitled "The Clinical Aspects of Cardiac Involvement (Right Auricular Tumor) in Idiopathic Hemorrhagic Sarcoma (Kaposi's Disease)" (Ann. Int. Med. **14**:314 [Aug.] 1940), records two necropsies in which the findings would appear on casual consideration to establish a new era in the history of Kaposi's disease—namely, primary Kaposi's disease of the heart without corresponding changes in the skin. However, the macroscopic and histologic descriptions and the solitary photomicrograph which is intended to illustrate the microscopic changes in one of the auricles are not convincing. I have been unable to obtain microscopic preparations in these cases.

or speckled or streaked by brown. The cut surfaces are smooth, and the substance is homogeneous. The consistency of the nodules is usually described as soft, sometimes as semifluid or mushlike. In the solid viscera they present much the same general appearance but may vary in diameter from 0.5 cm. to 7 or 8 cm. or more. Microscopically, the growths are oftenest described as composed of spindle cells.

Case 8.—M. Z., a Hungarian Jew aged 61, a dishwasher, was admitted to Bellevue Hospital Sept. 24, 1936 and died November 26. The patient said that for five months past he had been treated for "stomach trouble" which, he thought, resulted from two or three months' "near starvation." His symptoms included nausea, flatulence and abdominal cramps. At the time of admission he stated that his stomach was still intolerant of meats and that he had lost 20 pounds (9 Kg.) in weight in the past year. While in the hospital he often complained of cramplike pains in the region of the stomach and eructations of gas. The patient further stated that in the eleven years previous to admission he had noted numbers of lumps springing from the lower and upper extremities. The first lump was observed near the "second finger joint." Physical examination revealed marked emaciation. On both lower extremities, especially below the knees, there was an erythematous scaling rash. Both feet and hands were cyanotic, but the cyanosis diminished on elevation of the extremities. There were innumerable firm, rounded or oval purplish nodules, painless and nontender, distributed through the skin of both hands and feet. They were particularly numerous in the regions of the small joints. They were present also on the elbows. Over the small joints of the right hand they were so thickly set that the fingers were practically immovable. The fingers showed clubbing. The nodules varied in diameter from 1 to 12 cm. One or two of the smaller ones were superficially ulcerated. The patient stated that from time to time some of the nodules in the extremities disappeared completely or almost completely and reappeared in nearby situations. During his stay in the hospital several new lesions developed in the supraclavicular regions, groups of them in the lower part of the right thigh and one in the region of the left shoulder. The latter nodule attained a size of about 9 cm. in length and 7 cm. in breadth. One of those in the supraclavicular region measured 4 cm. in diameter. Those in the right thigh appeared as undulating bluish or dull purplish plateau-like formations. In addition there were several small lesions in the skin of the anterior thoracic wall and in that of the left arm. The spleen was palpated about 6 cm. below the left cosal margin. There were palpable lymph nodes in the left axilla, both groins and both popliteal spaces.

Analysis of the gastric contents showed absence of free hydrochloric acid in both the fasting and the alcohol test meal specimens. However, free hydrochloric acid was present following an injection of histamine. Blood was observed in all of the specimens. Occult blood was found in the stools.

The blood counts showed an average of 4,225,000 red cells and 17,000 white cells per cubic millimeter; the hemoglobin content was 67 per cent; polymorphonuclear neutrophils were 88 per cent, lymphocytes 10 per cent and eosinophils 2 per cent.

Roentgenographic examination revealed "atrophic arthritis involving the wrist joints and metatarsal and phalangeal articulations."

Necropsy.—The body was that of a well developed undernourished white man, 171 cm. in height, weighing about 50 Kg. The skin covering various parts of the body showed innumerable bluish red or purplish, moderately firm nodular or

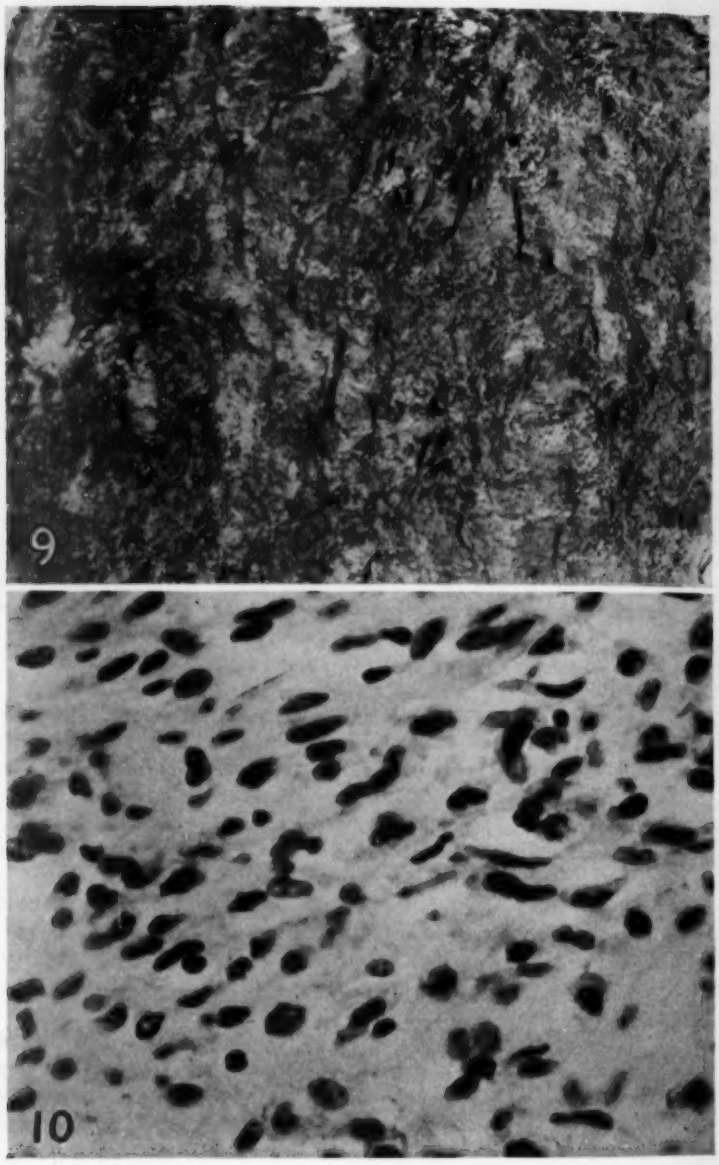


Fig. 9 (case 8).—Photograph of a microscopic section from a nodule in the skin showing almost complete replacement by hyalinized connective tissue. The darker streaks represent hemosiderin deposits. Eosin and Perl's reagent for iron; paraffin section; × 7.

Fig. 10 (case 8).—Photomicrograph of a section from an enlarged abdominal lymph node showing loosely distributed distorted fibroblasts, some displaying a tendency to arrange themselves in parallel formation. Hematoxylin and eosin; paraffin section; × 993.

plaquelike formations, the sizes and distribution of which were as described in the clinical history. The superficial lymph nodes in the inguinal regions formed nodular masses about 4 cm. in diameter. The fingers on both hands showed spindle-shaped hard knotlike deformities over the metacarpophalangeal joints.

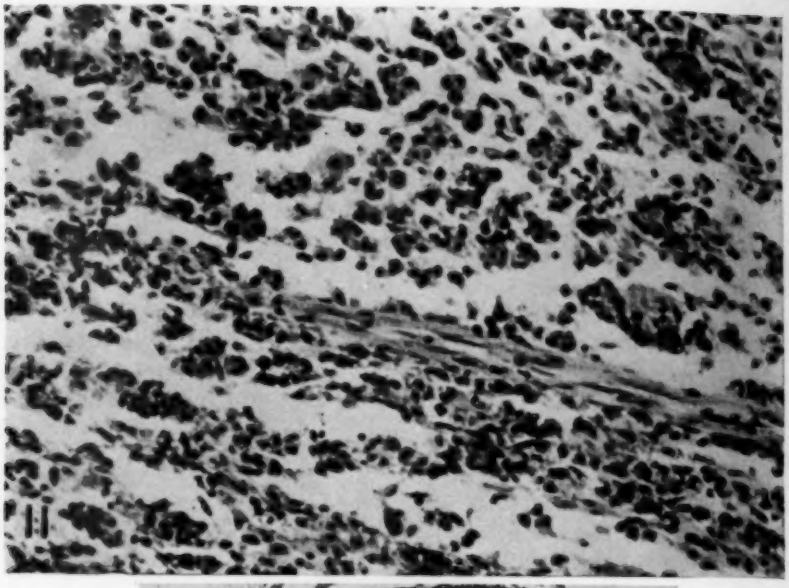
When the peritoneal cavity was opened 300 cc. of clear straw-colored fluid was released. The peritoneum showed innumerable firm beadlike nodules, averaging about 2 mm. in diameter, some of them closely apposed. Most of these nodules were yellowish white; some of them were dark brownish.

The heart weighed 250 Gm. The pericardium was smooth and glistening, and there was no excess of fluid. The posterior aspect of the left ventricular epicardium showed two whitish dome-shaped nodules, about 3 mm. in diameter; another nodule, 2 mm. in diameter, lay at the posterior-superior aspect of the interventricular septum, and two others of the same size were present in the right auricular epicardium. The endocardium was smooth throughout.

The right lung contained two whitish or faintly cream-colored circumscribed nodules, measuring 1 or 2 cm. in diameter. The tracheobronchial lymph nodes were markedly enlarged and were almost completely replaced by dense whitish tumor tissue. In the lower lobe of the right lung some of the bronchi were sheathed in grayish white homogeneous tumor tissue. The liver throughout its entire substance showed whitish or reddish nodules, measuring from 2 to 4 cm. in diameter, all of them sharply circumscribed. The spleen extended 7 cm. below the costal margin on the left side and weighed 1,000 Gm. On section the pulp was almost completely replaced by whitish or faintly pinkish circumscribed tumor nodules. The pancreas was practically completely replaced by whitish tumor masses. The entire posterior wall of the stomach from the lesser curvature downward was diffusely thickened to a depth of about 2 cm. by grayish tumor tissue, which in places protruded through the mucosa in the form of nodular masses of varying sizes. In the duodenum just below the pyloric ring two nodules were noted, each of which measured about 0.5 cm. in diameter. Dispersed throughout the submucosa of the rest of the intestinal tract were innumerable grayish nodules, measuring from 1.5 to 4 cm. in diameter. Many of these showed central ringlike depressions, the borders of which were reddish. The wall of the urinary bladder showed a few small rounded tumor masses, which were whitish or faintly cream colored. The mesenteric and retroperitoneal lymph nodes were greatly enlarged and were made up of discrete and conglomerate masses, measuring from 2 mm. to 3 cm. in diameter. The nodes were rather firm in consistency, and on section their cut surfaces varied in color. Some were brownish, others were whitish or flesh colored and others were pinkish or reddish. Various combinations of these three colors were found in some of the individual nodes.

Microscopic Examination.—A nodule removed from the skin showed a moderately richly cellular fibroblastic growth lying in the derma. It was surrounded by a thick layer of hyalinized connective tissue from which coarse bands extended into the growth and divided it into irregular lobules composed of a disorderly arrangement of spindle cells. However, perhaps the greater number of the fibroblasts were distributed in parallel formation. Scattered throughout the growth were innumerable small hemorrhagic extravasations. No pigment was detected. One gained the impression that this particular nodule was in process of connective tissue replacement.

A second nodule from the skin showed extensive overgrowth of intermingled coarse hyalinized connective tissue fibers arranged more or less parallel with one



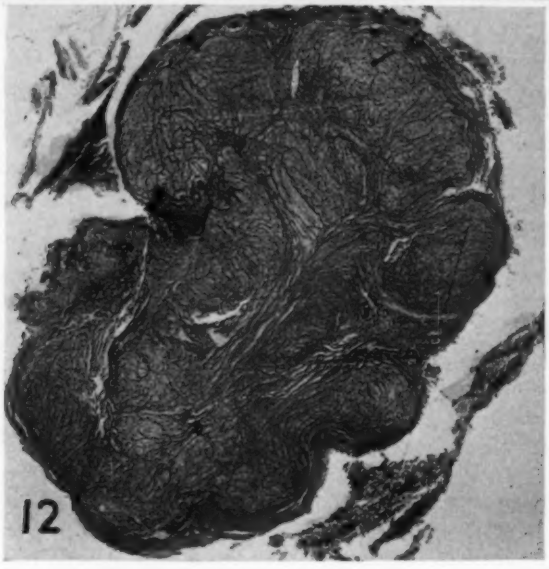


Fig. 11 (case 8).—Photomicrograph of a section from a metastatic tumor showing disorderly arrangement of the cells and polymorphism. Hematoxylin and eosin; paraffin section; × 259.

Fig. 12 (case 6).—Photomicrograph of a microscopic section from a single small nodule in the conjunctiva, showing trabeculation and a delicate argentophilic network. Silver impregnation; paraffin section; × 6.

another. Among them were a few fibroblasts, most of them curved, twisted or otherwise contorted, together with countless deposits of golden brown pigment granules (fig. 8).

A third piece of tissue removed from the skin revealed great numbers of broad intertwining and fused bundles of wavy collagenous connective tissue scattered between which were collections of granules giving the prussian blue reaction for hemosiderin. This nodule was practically completely replaced by scar tissue (fig. 9).

Innumerable tissues removed from the visceral deposits showed changes which were noticeably different from those in the skin but which among themselves were essentially alike, namely, vast numbers of loosely arranged cells of various shapes, sizes and chromatic richness, probably of fibroblastic origin. However, among them were numerous spindle cells which tended in some places to arrange themselves in loose parallel formation (fig. 10), in some places to form slender bundles of connective tissue, in others to occur without any attempt at orderly arrangement (fig. 11); in still others (fig. 11) they were separated by small collections of red blood cells. In addition, small hemorrhages were noted in moderate numbers throughout the tissue. In none of the many visceral sections examined were pigment deposits observed.

PATHOLOGIC CONSIDERATIONS

Except for the necropsy here recorded I have had occasion to observe the changes in Kaposi's disease only in excised nodules varying in diameter from 5 mm. to 4 cm. In 1 case a 5 mm. nodule was removed from the conjunctiva near the left cornea. The patient stated that his left eye had been troubling him for a period of three weeks. I therefore assumed that the nodule was young. In this nodule and in others of similar dimensions the histologic changes seemed to indicate that the disease from the beginning presents two competing factors—one characterized by a tendency to affect healing through overgrowth of connective tissue; the other showing a proclivity to perpetuate growth at an extraordinarily sluggish pace.

All of the cutaneous nodules in Kaposi's disease which I have had the opportunity to study were more or less well circumscribed by connective tissue. The smaller and presumably younger nodules were frequently sharply isolated by densely packed coarse fibrils which lay parallel with one another and took up those stains having an elective affinity for collagen; that is to say, they colored green with vert lumière, blue with aniline blue or red with acid fuchsin. In tissues impregnated by silver the same fibrils were deep brownish. Trabeculae entered the nodules at irregular intervals, dividing them into lobules of different sizes. The trabeculae divided into fibrils, which were soon lost in the deeper parts of the growth. In addition to all of these, innumerable delicate fibrils arranged in a complex network were brought out in tissues impregnated with silver and were jet black (figs. 12, 13, 14). Lying in the meshes of this network were variable numbers of rather plump spindle cells which presented a light brownish appearance. Each

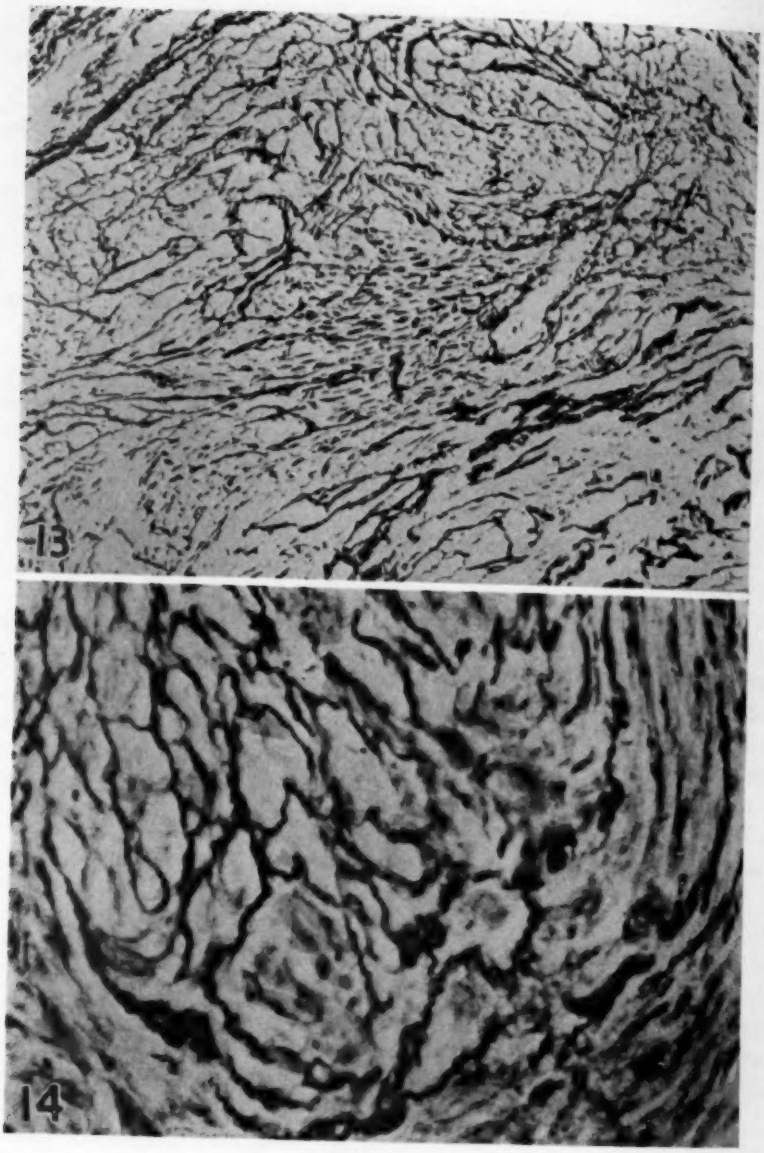


Fig. 13 (case 6).—Photomicrograph of a section from a small nodule in the skin showing argentophilic reticulum supporting fibroblasts; some of the latter are cut lengthwise and others cross-wise. Silver impregnation; paraffin section; × 112.

Fig. 14 (case 6).—Photomicrograph of a section from the same nodule as figures 12 and 13, showing the granular remains of fibroblasts in a coarsening argentophilic reticulum network that is beginning to arrange itself in closed formation. Silver impregnation; paraffin section; × 660.

was provided with a nucleus which occupied almost all of the spindle. In some instances they were arranged parallel with one another in columns so that in a given microscopic field they looked not unlike a spindle cell sarcoma in miniature (fig. 13). In other places their structure was partially or completely obscured by granular changes incidental to early necrosis. As necrosis progressed, the spindle cells tended to disappear, and the individual argentophilic fibrils contracted into wavy folds and took on a brownish-black color, eventually becoming thickened and arranged in closed formation (fig. 14).

For further descriptive purposes the nodule may be divided into two parts: (*a*) One part is composed of variable numbers of spindle cells which lie parallel with one another in interlacing bundles. The derivation of these cells has not been demonstrated. They have been variously described as coming from the fibromuscular walls of small blood vessels or from nerve sheaths or from the endothelium of capillaries. In the examination of the tissues available to me I have not been able to trace them to any one of these sources. It is my impression that they are derived from those spindle cells which are normally present in the papillary, or upper, layer of the derma. Whatever their origin may be, the cells in question when stained with hematoxylin and eosin present the structural attributes of fibroblasts. Between them are fibrils which are colored green with the vert lumière of Masson's stain and reddish with the acid fuchsin of Van Gieson's stain. Both of these stains are known to have an elective affinity for mature collagenous fibrils. No cell other than the fibroblast is known to produce collagenous fibrils. It follows as a logical inference that the spindle cells in question are fibroblasts. (*b*) The other part of the nodule in Kaposi's disease is composed of innumerable slitlike "sinusoids" lying between the fibroblasts. In the sinusoids are red blood cells. The latter may be seen in twos or threes, or they may occur as large extravasations lying outside the sinusoids. They are evidently derived from the capillary vessels of the nodule as a result of rupture, most probably traumatic. Eventually the liberated red cells disintegrate, leaving collections of granules which may be identified as hemosiderin by their yellowish brown color and by the prussian blue reaction.

In short, by the use of simple differential staining methods it has been shown, I believe, that the unit of growth in Kaposi's disease is a fibroblast. The fibroblasts appear to produce two sorts of reticulum fibrils. One sort is immature and becomes black when impregnated with silver. It does not take up the ordinary dyes that are elective for collagen. The other sort of fibril is mature and takes the stains elective for collagen by the methods of Masson and Van Gieson. The function of the argentophilic reticulum is a dual one. In the young nodules of Kaposi's disease it is arranged as a fine network for the mechanical

support of the fibroblasts; at other times it participates in the process of healing by reenforcing the mature collagenous fibrils, in this way aiding in the replacement of cells which have undergone necrosis and disappeared. The mechanism of healing through the participation of the argentophilic system of reticulum fibrils is not peculiar to Kaposi's disease. It may also be demonstrated in certain productive inflammatory lesions, notably in the young reticulated argentophilic form of the epithelioid tubercle.[6]

In a patient with Kaposi's disease whom I saw at Bellevue Hospital nodules and plaques were extensively distributed through the skin of the lower half of both legs, over the dorsal surfaces of both feet and to a lesser extent over the inner aspects of the plantar surfaces. The lesions presented a dull violaceous hue and were relatively smooth, flattened and noticeably firm in consistency. There were no signs of recent hemorrhage in any of them. However, in many of the small flattened nodules and at the edges of the plaques for a distance of about 5 or 6 mm. were areas of dull greenish discoloration, while scattered through the substance of the plaques were similarly discolored areas. The presence of the greenish hue suggested the deposition of a second variety of pigment.

Sections from the edge of one of the greenish pigmented plaques were treated by Perl's reagent. In addition to the prussian blue visible in the deposits of hemosiderin there was apparently another pigment which seemed to be faintly greenish. It is sometimes difficult to distinguish the lighter shades of blue and green, especially in microscopic preparations, and since I found myself handicapped in this particular I asked one of the interns in pathology, who is color blind for green, to look at several sections which had been treated with Perl's reagent. The intern in question has no conception of the color green. All things which are green to the person with normal eyes are gray to him. He immediately identified the prussian blue but stated that there was another substance in the section which appeared to him to be gray. In untreated sections from the same paraffin blocks innumerable golden brown deposits of hemosiderin were apparent, but there was no indication of a green pigment such as one might expect, for example, if biliverdin or iron phosphate were present. I assume, therefore, that if there is a second pigment in the lesions of Kaposi's disease, its nature has not been disclosed.

In Kaposi's disease those nodules are most deeply pigmented in which there has been destruction of blood, since the pigment, hemosiderin, is a derivative of hemoglobin. The pigment is found almost exclusively in those nodules where there has been replacement by con-

6. Symmers, D.: Arch. Path. **31**:304, 1941.

nective tissue either in the form of small scars or as more nearly complete overgrowths. The pigment lies between the connective tissue bundles (fig. 8). In other words, the stimulus for overgrowth of connective tissue in those nodules which spontaneously regress is probably to be sought in the sudden escape of blood followed by destruction and regeneration of fibroblasts and, finally, by replacement fibrosis. Practically all of the surface nodules, more especially those of the skin and of the gastrointestinal tract, are exposed to mechanical injury. The blood vessels in these lesions are simple endothelial channels and may rupture under slight provocation. The ensuing extravasations are composed of red blood cells which are well formed, stain brightly, appear to be rich in hemoglobin and do not clot but lie free in closed tissue spaces. In these circumstances one is apt to inquire if failure to clot is due to low prothrombin content. I am not aware if the prothrombin content of the blood in Kaposi's disease has been determined, but if prothrombin is not present in adequate quantities, the administration of vitamin K might be considered as a therapeutic measure on the supposition that it would be followed by clotting and by organization of the clot and thus bring about healing of the nodule. I have not had an opportunity to apply these theoretic considerations, since no patient with Kaposi's disease is at present available for this method of treatment, but I purpose to do so if occasion should present itself.

RÉSUMÉ AND CONCLUSIONS

The unit of growth in the cutaneous nodules of Kaposi's disease is the fibroblast. The growth springs from those fibroblasts which lie loosely in the papillary, or superficial, layer of the derma, where normally they are relatively well nucleated and produce collagenous fibrils in small numbers, rather than from the fibroblasts in the deeper, or reticular, layer of the derma, where normally they are poorly nucleated and produce collagenous fibrils in large numbers arranged in thick bundles. The mucosal growths most probably arise from the subepithelial fibroblasts, although I have no definite evidence to offer on this phase of the subject or on the origin of the presumably nonmetastatic growths in the solid viscera.

The fibroblast determines the growth behavior of each of the phases by which Kaposi's disease may be characterized: (*a*) The fibroblast may produce young argentophilic collagenous fibrils which reenforce the mature collagenous bundles in such manner as to aid in the process of healing. (*b*) The fibroblast may maintain a low, almost stagnant capacity for growth over a long number of years. (*c*) The fibroblast may suddenly assume active malignant properties and terminate life by widespread destruction of tissues through the process of metastasis.

Histologic study of the younger growths shows that from the outset two opposing factors are operative—one tending to bring about replacement of the nodules by the production of collagenous fibrils, the other tending to maintain the capacity of the nodule to grow, but at an extremely slow rate.

Excluding such incidental features as hemorrhagic extravasations between the fibroblasts and deposits of pigment following destruction of red blood cells, the histologic appearance of the well developed growths in Kaposi's disease is scarcely to be distinguished from that of the familiar spindle cell sarcoma. Whereas the familiar spindle cell sarcoma almost always grows rapidly and without restraint, the progress of the spindle cell growths of Kaposi's disease is retarded almost to the point of stagnation. In other words, the growths in Kaposi's disease are histologically malignant but clinically benign.

The process of spontaneous regression and healing in the cutaneous lesions of Kaposi's disease is probably initiated by the sudden release, traumatic or otherwise, of large numbers of red blood cells followed by injury to or destruction of neighboring fibroblasts, then by regeneration of fibroblasts and finally by connective tissue replacement.

The smaller and presumably younger nodules in Kaposi's disease are permeated by a complex network of argentophilic reticulum which serves primarily as a mechanical support for the fibroblasts.

INCIDENCE OF OCCULT ADENOCARCINOMA OF THE PROSTATE

AFTER FIFTY YEARS OF AGE

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AND
ALFRED ANGRIST, M.D.
JAMAICA, N. Y.

The incidence of carcinoma is always higher for the pathologist than for the clinician. This discrepancy is more outstanding in regard to prostatic carcinoma than in regard to other forms of cancer, since the tumors are often too small to be felt or seen clinically. The difficulty in diagnosis exists often at autopsy, with the entire prostate excised and sectioned, as many of the carcinomatous tumors are small and can be found only on careful microscopic examination.

Different authors have reported the incidence of occult carcinoma with figures ranging from 13.9 to 29.4 per cent. This discrepancy can be accounted for by the variation in the methods of investigation. Barringer [1] found carcinoma in 17.4 per cent of prostates showing adenomatous hyperplasia. Myers [2] reported an incidence of 29.4 per cent in tissue removed at prostatectomy. Kahler [3] claimed the incidence to be 25 per cent in the prostates of men over 60 years of age and 13.9 per cent in all prostates examined. Rich,[4] examining one slide of each prostate, claimed an incidence of 14 per cent in the prostates of men over the age of 50, in a series of 292 prostates. Moore,[5] examining the entire prostate, found carcinoma in 20.5 per cent of those from 229 men over 50 years of age. His entire series, from men ranging in age from 21 to 90 years, shows an incidence of 16.7 per cent.

METHOD OF STUDY

The material used in the present study represents two series of cases. The first group of cases covers the routine autopsies performed at the Queens General Hospital over the four year period from 1935 to 1939, a total of 2,024. During this period 605 autopsies were performed on men over the age of 50. In 364

From the Department of Pathology, Queens General Hospital.

1. Barringer, B. S.: Am. J. Roentgenol. **37**:49, 1937.
2. Myers, G. M.: Colorado Med. **34**:248, 1937.
3. Kahler, J. E.: J. Urol. **41**:557, 1939; Proc. Staff Meet., Mayo Clin. **13**:589, 1938.
4. Rich, A. R.: J. Urol. **33**:215, 1935.
5. Moore, R. A.: J. Urol. **33**:224, 1935.

of these cases only random sections of the prostate were examined microscopically. The number of sections in each instance varied from 1 to 15. The larger number of sections was taken in cases in which malignancy was suspected on gross examination at the autopsy table, or represented additional sections cut to confirm or disprove a suspicion as to malignant change in an area in an initial routine microscopic slide. The second group consists of 50 consecutive cases in which men over 50 years of age died of various causes other than carcinoma of the prostate and a complete study of the prostate was made. In this group the diagnosis of malignant growth of the prostate was *not* made clinically or at the time of autopsy. The prostate was removed completely and fixed in toto in 4 per cent formaldehyde solution for at least three days. The fixed organ was sectioned coronally and then divided into lateral and posterior lobes, including the median lobe with the posterior lobe (fig. 1).

CRITERIA USED IN DIAGNOSIS OF CANCER OF THE PROSTATE

The histologic criteria for the diagnosis of carcinoma of the prostate differ somewhat from those ordinarily applied elsewhere in the body.

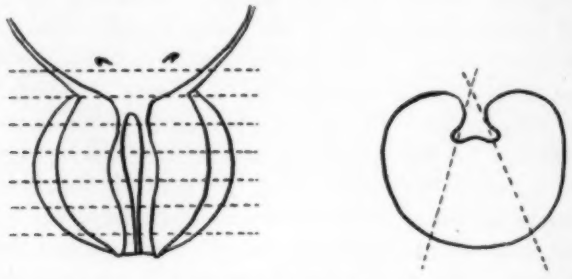

Fig. 1.—Diagram showing method of sectioning the prostate.

In proved cases of carcinoma of the prostate with regional infiltration and metastasis to lymph nodes or to bone, there is usually present an outstanding preservation of glandular differentiation. Anaplasia and mitotic figures are of little value in determining malignancy, as such features are scarce and frequently absent. The diagnosis is best made with the low power objective of the microscope, for then the relationship of the carcinoma tissue can be seen, with its inherent histologic, rather than cytologic, disorientation. The area of invasive glandular tumor tissue stands out in contrast to the surrounding oriented residual normal acinar tissue, with corresponding duct structures. The carcinoma is recognized by nests of small acini invading the stroma between the intact lobules of the gland. The haphazard arrangement exhibits no tendency to orientation with included duct structures, and this constitutes the outstanding histologic feature of the common form of adenocarcinoma of the prostate. Invasion of regional nerve structures, or definite extension into lymphatic spaces or extension into the capsule of the gland, often accompanies this appearance. The cells may be

hyperchromatic with a proportionately large nucleus and with only occasional loss of nuclear polarity. A distinct scirrhous tendency with tubular arrangement of the elements, such as is found in scirrhous cancer of the breast, is found rather frequently. A frankly medullary carcinoma was seen only in 2 of the 50 controlled cases. Transitions were seen from a precancerous hyperplasia to adenocarcinoma to areas showing a distinct scirrhous reaction or frank medullary appearance (figs. 2 and 3).

RESULTS AND ANALYSIS

Analysis of the first group of 364 cases reveals the incidence of adenocarcinoma to be 14.8 per cent. In 9.9 per cent of the instances of carcinoma the tumors were small and classified as occult. When the fact is considered that in most cases only one or two random sections were taken of each prostate, the incidence of carcinoma in this material is high. Additional sections were taken only in cases in which carcinoma was believed to be present in the gross specimen or in which outstanding and somewhat atypical adenomatous hyperplasia was demonstrated.

Attempts were made to correlate prostatic carcinoma with other lesions in the prostate. No positive correlation was found, except that carcinoma occurred more frequently in atrophic areas of the prostate. Carcinoma of the prostate seems to be more frequent in men who have carcinoma elsewhere, though no definite statement is permissible, because of the limited number of cases. When carcinoma is found in hyperplastic glands, it usually does not occur in the adenomatous nodules but in the intervening atrophic areas. A similar dissociation is found for acute and chronic prostatitis. This may have some practical clinical application, in that the ordinary prostatectomy removes hypertrophied nodular prostatic areas and leaves peripherally placed compressed encapsulating atrophic prostatic tissue. The age of the patient is the most significant factor in correlation, the incidence increasing and reaching a peak in the ninth decade.

The findings in this first group of cases prompted the second study. In order to determine more accurately the incidence of occult carcinoma, the entire prostate was examined in the second series of 50 consecutive cases of patients over 50 years of age. Cases were not selected as to cause of death, and no attempt was made to select prostates with areas arousing suspicion. The general size of the prostate was noted and, to evaluate the accuracy of the gross diagnosis, an attempt was made to pick out areas in the gross specimen which suggested induration or other gross criteria of carcinoma. In only 1 case of the entire series of 50 was the area suspected of carcinoma found to have been chosen accurately. In this instance there was a rather large and anaplastic growth

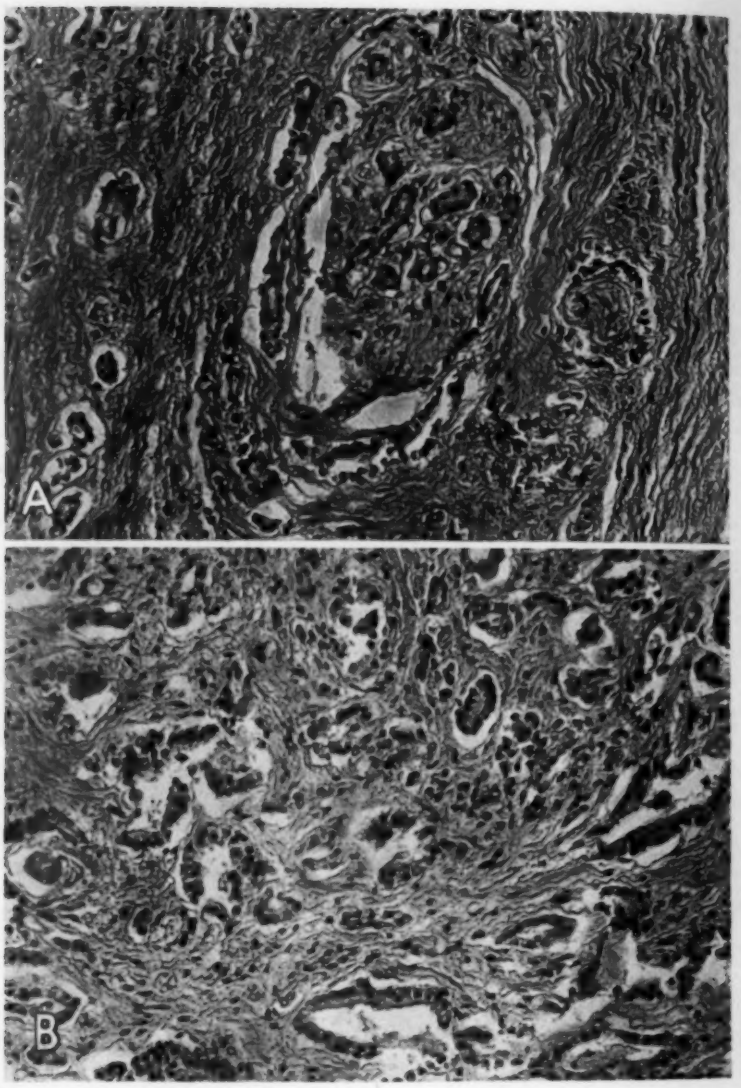

Fig. 2.—*A*, definite miniature adenocarcinomatous lesion with invasion into nerve structure and perineural lymphatic. *B*, adenocarcinoma showing rather hypertrophic atypical glandular tissue with loss of orientation to duct structures.

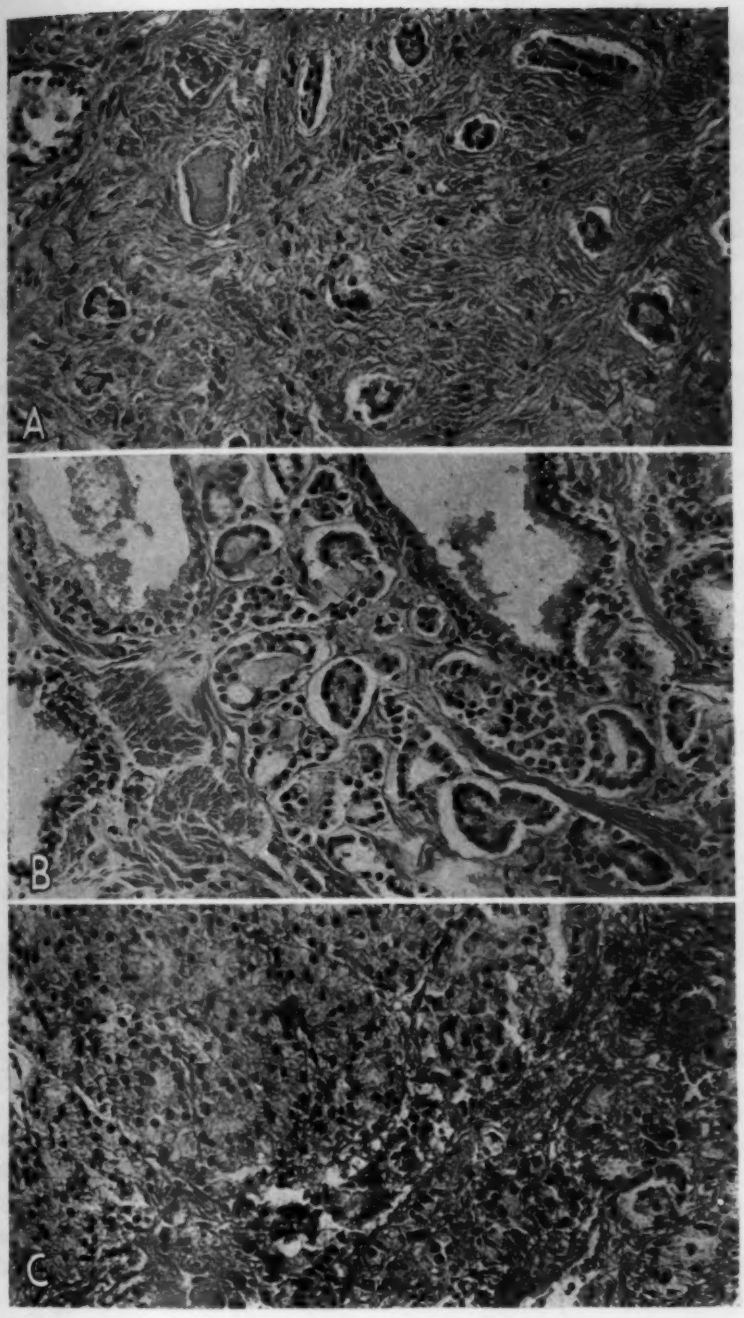

Fig. 3.—*A*, preserved glandular carcinoma with considerable desmoplasia. *B*, hyperplastic glands showing lack of orientation to duct structures and suggesting invasion of stroma. The lesion is considered precancerous and equivocal. *C*, compact glandular carcinoma with medullary features.

and the gross criteria were obvious. Usually the firm, solid, white areas arousing suspicion were found to be myomatous zones. The minute yellow areas, ordinarily accepted as pertinent gross evidence of carcinoma, were found to represent adenomatous hyperplastic nodules, with and without necrotic foci.

Using the criteria which we have outlined in a foregoing section, we found carcinoma in 23 of the 50 prostates, an incidence of 46 per cent.

TABLE 1.—*Series of Three Hundred and Sixty-Four Cases in Which Random Sections of the Prostate were Examined at Autopsy*

Age	Patients	Inflammation or Hyperplasia of Prostate		Total Carcinoma		Occult Carcinoma of Prostate	
		Number	Per Cent	Number	Per Cent	Number	Per Cent
50-59	140	116	83	12	8.6	9	6.4
60-69	124	99	80	18	14.5	10	8.1
70-79	81	71	88	19	23.4	13	16
80-89	19	17	90	5	26.3	4	21
Total	364	303	83	54	14.8	36	9.9

	Number	Per Cent
Carcinoma found somewhere at autopsy	145	40
Carcinoma of prostate with no other carcinoma	44	12.1
Carcinoma of prostate with carcinoma elsewhere	10	2.7
Carcinoma elsewhere without carcinoma of the prostate	92	25.3

TABLE 2.—*Series of Fifty Cases in Which Prostate Was Sectioned Completely*

Age	Patients	Occult Carcinoma of Prostate		Hyperplasia of Prostate		Inflammation of Prostate	
		Number	Per Cent	Number	Per Cent	Number	Per Cent
50-59	19	8	42	13	68	16	84
60-69	21	8	38	16	76	19	90
70-79	9	6	66	7	78	9	100
80-89	1	1	100	1	100	1	100
Total	50	22	46	37	74	45	90

	Per Cent
Carcinoma found somewhere at autopsy	56
Carcinoma of prostate with no other carcinoma	32
Carcinoma of prostate with carcinoma elsewhere (one metastatic oat cell carcinoma of lung to prostate)	14
Carcinoma elsewhere without carcinoma of the prostate	10

In 9 of these 23 glands the carcinoma was in the lateral lobes, and in 4, in the posterior lobe. In the remaining 10 the neoplasm either was too large to allow determination of the site of origin or occurred at the site of junction of the lateral and posterior lobes, invading both. This differs from the widely accepted concept that carcinoma usually originates in the posterior lobe. As in the previous series, adenocarcinoma seems to start in the atrophic areas and is occasionally multicentric. In most prostates, only one or two slides of the entire series show adenocarcinoma. The remaining portions of the gland, partic-

ularly adjacent areas, often show sufficient atypism and irregularity in the size of acini and tendency to invade to warrant consideration of precancerous changes. This suggests that some physiologic stimulus was being applied to the entire gland and was affecting the regional tissue to a varying degree. As in the first general series, no correlation was found between prostatic carcinoma and other lesions of the prostate. The cases are far too few, however, to allow one to place any significance on this negative finding. The incidence increased with age, reaching a peak in the eighth decade.

Despite the limited number of cases, the incidence of prostatic carcinoma is undoubtedly high for unselected material. Inasmuch as so few of the tumors ever reach sufficient size to be a factor in the cause of death, they must be very slow-growing neoplasms. The probability is that they exist for many years, without causing symptoms and without metastases. It must be emphasized, however, that the histologic appearance and criteria of malignancy used in the identification of occult tumors are identical with those found in definite adenocarcinoma of the prostate, confirmed by biologic invasion and metastasis to nodes and bone.

SUMMARY

The incidence of occult adenocarcinoma of the prostate is high. By examination of random sections taken from prostates of 364 men over 50 years of age, 9.9 per cent of these prostates were found to contain occult adenocarcinoma. In this series 4.9 per cent of the patients died of extensive prostatic adenocarcinoma with metastases, making a total incidence of 14.8 per cent.

In a series of 50 unselected consecutive autopsies on men over 50 years of age the prostate was examined completely, and the incidence of occult carcinoma was found to be 46 per cent. In this group, prostatic carcinoma was not the cause of death in any case. Metastases were not found and the diagnosis of carcinoma was not made at the time of autopsy.

The incidence of occult carcinoma increases with age. Although inflammation and adenomatous hyperplasia likewise increase with age, the lesions could not be definitely correlated. Miniature carcinoma usually occurs in an area of atrophy. Carcinoma is found in the lateral lobes or at the junction between lateral and posterior lobes more often than in the posterior lobe proper. Because of the high incidence, the small size of the tumors and the fact that metastases are not found in these cases, it must be assumed that the tumors are very slow growing and seldom become a factor in the cause of death.

EFFECTS OF CONTINUED ADMINISTRATION OF SULFATHIAZOLE AND SULFAPYRIDINE IN MONKEYS

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The early experimental background [1] for the preliminary clinical investigations on sulfathiazole [2] (2-[paraaminobenzenesulfonamido]-thiazole) dealt primarily with the chemotherapeutic and general pharmacologic properties of the drug. The present communication deals solely with a comparison of the effects produced by continued administration of doses varying from ten to two hundred times the usual therapeutic range of doses of sulfapyridine (2-[paraaminobenzenesulfonamido]-pyridine) and sulfathiazole to a series of normal monkeys. This study, simulating clinical therapeusis, is a logical extension of the work reported by Molitor and his colleagues on sulfapyridine [3] and offers an experimental basis for a comparison of these two important drugs as to relative toxicity when administered, save for the massive doses, under conditions approaching those encountered in clinical practice.

MATERIAL AND METHODS

The material consisted of a series of 27 normal adult Macacus rhesus monkeys of both sexes. The animals were kept in individual cages and fed on a normal

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1. Cooper, F. B.; Gross, P., and Lewis, M.: Proc. Soc. Exper. Biol. & Med. **42**:421, 1939. McKee, C. M.; Rake, G.; Greep, R. O., and Van Dyke, H. B.: ibid. **42**:417, 1939. Barlow, O. W., and Homburger, E.: ibid. **42**:792, 1939. Lawrence, C. A.: ibid. **43**:92, 1940. Rammelkamp, C. H., and Keefer, C. S.: ibid. **43**:664, 1940.

2. Flippin, H. F.; Schwartz, L., and Rose, S. B.: Ann. Int. Med. **13**:2038, 1940. Finland, M.; Lowell, F. C., and Strauss, E.: New York State J. Med. **40**:115, 1940. Spink, W. W., and Hansen, A. E.: J. A. M. A. **115**:840, 1940. Helmholz, H. F., and Larsen, N.: Proc. Staff Meet., Mayo Clin. **15**:651, 1940. Carroll, G.; Kappel, L., and Lewis, B.: Weekly Bull. St. Louis M. Soc. **35**:48, 1940.

3. Molitor, H., and Robinson, H.: Arch. internat. de pharmacodyn. et de thérap. **62**:281, 1939.

mixed diet. Each drug was administered as a 20 per cent suspension of a finely ground (100 mesh) powder in evaporated milk. All medication was by stomach tube. The daily dose was divided into three equal portions, which were administered at eight hour intervals, viz., 8 a. m., 4 p. m. and 12 midnight. A blood sample was removed four hours after the morning dose and the blood concentration of the drug was estimated by a slight modification [4] of Marshall's [5] methods.

RESULTS AND COMMENT

During the course of medication, a characteristic symptom complex developed in all animals save those on the low dose of sulfathiazole. Anorexia, nausea with or without vomiting, and diarrhea became apparent in most of the animals. The anorexia became increasingly severe, and in those experiments which terminated with fatal reactions it was complete for the last day or so prior to death, which was invariably preceded by clonic convulsions.

Gross hematuria was observed in practically all animals of the sulfapyridine series but was rarely observed in those of the sulfathiazole series. The nature of the experimental material, however, precluded the possibility of any precise quantitative analysis of the urinary constituents, but the gross picture presented in experiments which were ended by fatalities was one of marked oliguria or complete anuria in the terminal phases of the medication. The validity of this impression was corroborated by the sudden progressive elevation of the blood concentration of the drug during this phase.

Tables 1 and 2 give brief synopses of the protocols and pathologic observations for all animals in the series.

At a dose level of 0.5 Gm. per kilogram per day 3 monkeys on sulfapyridine died on the fourteenth, fourteenth and twenty-fifth days of medication, respectively. In every instance death was preceded by

4. We have obtained satisfactory results by employing a combination of the methods described by Marshall: The blood sample is added directly to 19 volumes of ethyl alcohol and centrifuged. Any turbidity which may occur in the centrifugate may be readily removed by treating the supernatant fluid with small quantities of filter-cel [4a] before filtering. The filtrate is then acidified and treated with sodium nitrite. The excess of sodium nitrite is removed by the addition of ammonium sulfamate. The diazonium salt thus formed is coupled with N′ (naphthyl)-ethylenediamine dihydrochloride, and the color developed is compared with those of suitable standards of proper concentration in a photoelectric colorimeter.

4a. Filter-cel (Johns-Manville Company) is a commercial preparation of a diatomaceous earth, an inert material formed from deposits of marine plankton diatoms and composed primarily of the following species: Triceratium, Auliscus, Thalassiothrix, Synedra Nitaschia, Coscinodiscus, Stictodiscus, Chactoceros.

5. Marshall, E. K., Jr.; Emerson, K., and Cutting, W. C.: J. A. M. A. **108:** 953, 1937. Marshall, E. K., Jr., and Litchfield, J. T.: Science **88**:2273, 1938. Marshall, E. K., Jr., and Bratton, W.: J. Biol. Chem. **123**:537, 1939.

hematuria, anorexia and coma. Postmortem examination of the renal tracts of these animals showed masses of crystalline material of greater or less extent with or without gross urolithiasis and varying degrees of renal obstruction. Crystalline material was visible in the collecting tubules. Uroliths, together with crystals, were also found in the ureters and the bladders. Grossly, the uroliths showed sharp, needle-like spicules of crystalline material embedded in a matrix of bloody amorphous granular debris. These were well formed masses which could easily be handled without breaking. The crystalline material, which comprised only a small part of the urolith, was found to be composed of both the acetylated and the free form of sulfapyridine, the former predominating.

Histologic examination of various organs indicated the presence of toxic splenitis to a moderate degree, associated with slight erythrophagocytosis and very slight parenchymatous degenerative change in the liver. The principal lesion was in the kidneys: These were congested and edematous. There was a marked increase in the size of the capsular spaces, which were dilated and filled with an albuminous material; the glomerular tufts were constricted, and there was a slight increase in the number of nuclei present. The convoluted tubules were dilated, and the epithelial cells showed marked parenchymatous degeneration with patchy areas of fatty degeneration. The dilatation of the collecting tubules was more marked, and the epithelium showed focal areas of fatty degenerative change with necrosis and desquamation of the cellular elements and sometimes the presence of epithelial casts. These local foci of severe degenerative change appeared to be the result of an irritative process of a mechanical nature. The diffuse degenerative process seen in the epithelium of the convoluted tubules appeared to be a manifestation of a toxic process and may be attributed to one or both of two factors, viz.: (*a*) toxic effect of the drug; (*b*) generalized toxemia resulting from impairment of the elimination of toxic metabolites as a result of inefficient renal function. The most severe lesions occurred at the renal papillae and in the renal pelvis, which was the site of an acute exudative inflammatory reaction associated with submucosal hemorrhages and desquamation of the epithelial elements. The inflammatory reaction in the tubules centered about an acellular mass of pale blue-staining strandlike material which had the appearance of a weblike matrix, from which some crystalline substance, presumably crystals of the drug, had been washed away.

The administration of a corresponding dose (0.5 Gm. per kilogram per day) of sulfathiazole to another group of 3 animals produced no untoward reactions other than diarrhea, and all survived twenty-eight days of medication without showing any ill effects. Two of these ani-

TABLE 1.—*Effect of Continued Oral Administration of Sulfapyridine to Monkeys*

Monkey	Dose (Gm. per Kg. per Day)	Duration of Medication (Days)	Symptoms and Day of Appearance	Day of Death	Pathologic Observations
25	0.5	14	Hematuria (4), anorexia (8)	14	Gross: Kidneys congested and slightly edematous; pelves and ureters dilated; bladder contracted. On section, crystalline material present in many tubules. Pelves hemorrhagic and contain white, insoluble crystals. Ureters obstructed at bladder by impacted bloodstained crystalline casts. Bladder hemorrhagic and contains blood-stained urine and crystals. Microscopic: Kidneys edematous. Parenchymatous and fatty degeneration of tubular epithelium. Many glomerular capsules dilated by fluid, with compression of tufts. Collecting tubules contain small amounts of fluid. Pelvic epithelium thickened.
26	0.5	24	Hematuria (4), anorexia (17), glossitis (18)	25	Gross: Kidneys markedly enlarged, pale and edematous; pelves and ureters greatly dilated; bladder contracted. On section, pelves hemorrhagic and contain numerous sharp needle-like crystals 0.1 to 0.5 cm. long. Blood-stained urine with suspended crystals in ureters, which are obstructed at bladder by hard blood-stained crystalline casts. Bladder inflamed and hemorrhagic and contains blood-stained urine and a large quantity of sharp, spiculated grayish white uroliths. A small mucosal tear is present. Microscopic: Kidneys markedly edematous, especially tubules. Capsular spaces of glomeruli dilated by fluid, often with compression of tufts. Marked parenchymatous and moderate fatty degeneration of tubular epithelium with foci of necrosis. Epithelium of collecting tubules also degenerated, often desquamated with accumulation of epithelial casts in lumens. Thrombi present in many venules. Pelves inflamed and hemorrhagic and contain amorphous granular debris, desquamated tubular epithelium and rare neutrophils and red blood cells.
27	0.5	8	Hematuria (4), ataxia (8), convulsions (8), anorexia (8)	14	Gross: Kidneys slightly edematous; pelves and ureters dilated; bladder contracted. On section, needle-like crystals present in large collecting tubules. Pelves filled with blood-stained urine, in which are fine, needle-like crystals. Mucosa hemorrhagic and inflamed. Left ureter partly obstructed by impacted blood-stained, crystalline casts. No obstruction found in right ureter. Bladder inflamed and contains blood-stained urine, crystals and formed casts. One large cast, 1.5 by 0.3 by 0.3 cm. in size probably came from right ureter. Microscopic: Kidneys edematous. Many convoluted tubules contracted and separated from basement membranes, appearing as collapsed epithelial cylinders lying free in wider spaces. Marked parenchymatous and fatty degeneration of tubular epithelium with foci of necrosis. Glomeruli compact, often more cellular than usual and frequently surrounded by dilated, fluid-filled capsular spaces. Marked degeneration of collecting tubular epithelium with desquamation into lumens. Near papillae these cell accumulations appear to be casts. Many venules contain fibrinous thrombi. Pelves inflamed and hemorrhagic and contain fluid, epithelial cells and red blood cells. One portion of pelvic space obliterated by adhesions.

TABLE 1.—*Effect of Continued Oral Administration of Sulfapyridine to Monkeys—Continued*

Monkey	Dose (Gm. per Kg. per Day)	Duration of Medication (Days)	Symptoms and Day of Appearance	Day of Death	Pathologic Observations
1	1.0	7	Vomiting (1), hematuria (5), anorexia (7)	8	Gross: Kidneys swollen; pelves and ureters dilated; bladder moderately distended. On section, crystalline material present in collecting tubules. Pelves congested and together with ureters contain uroliths and blood-stained crystalline material, some aggregated into casts which obstruct ureters, right at pelvic brim, left at bladder. Bladder inflamed and hemorrhagic, and filled with blood-stained urine, granular material and many uroliths, the largest 0.5 cm. in diameter. Microscopic: Kidneys edematous, especially convoluted tubules. Capsular spaces dilated by fluid; capillary tufts compressed. Glomeruli congested, some more cellular than normal. Tubular epithelium shows parenchymatous and fatty degeneration. Papillae focally hemorrhagic and inflamed. Fibrinous thrombi in many venules. Pelves inflamed and contain serosanguinous material.
2	1.0	28	Vomiting (8), abdominal distention (11), hematuria (21), anorexia (25)	30	No postmortem examination (putrefactive changes)
3	1.0	7	Hematuria (3), anorexia (5)	8	Gross: Kidneys swollen; pelves and ureters markedly dilated; bladder normal. On section, collecting tubules contain fine, needle-like crystals. Pelves inflamed and hemorrhagic, and contain blood-stained urine and fine crystals. Left pelvis also contains large hard calculi 0.3 to 0.7 cm. in diameter. Ureters obstructed at bladder by impacted crystalline masses, which cannot be moved by pressure from above or below. Bladder inflamed and contains bloody urine, masses of crystalline material and amorphous granular debris. Microscopic: Kidneys congested and edematous. Extreme parenchymatous and fatty degeneration of tubular epithelium. In places changes are those of severe nephrosis. Capsular spaces dilated by fluid; capillary tufts compressed. Epithelium of collecting tubules degenerated, disintegrated and desquamated, forming castlike accumulations near papillae. Acute periarteritis and periphlebitis and endophlebitis, with thrombi in some venules. Pelves focally hemorrhagic.
4	2.0	16	Hematuria (4), anorexia (5)	17	Gross: Kidneys swollen and pale; pelves and ureters markedly dilated; bladder not remarkable externally. On section, small, needle-like crystals present in collecting tubules. Pelves inflamed and hemorrhagic, and together with ureters contain spiculated uroliths and blood-stained urine. Ureters obstructed at bladder by impacted crystalline granular blood-stained masses, which cannot be dislodged readily. Bladder inflamed and hemorrhagic and contains blood-stained urine, crystals and one small ureteral cast.

TABLE 1.—*Effect of Continued Oral Administration of Sulfapyridine to Monkeys—Continued*

Monkey	Dose (Gm. per Kg. per Day)	Duration of Medication (Days)	Symptoms and Day of Appearance	Day of Death	Pathologic Observations
					Microscopic: There is advanced parenchymatous and fatty degeneration of tubular epithelium. Tubules distended with fluid. Glomeruli small, compact, sometimes congested. Many capsular spaces dilated with fluid. Collecting tubules generally contracted, showing shrinkage, degeneration and desquamation of epithelium, often with acute inflammation. Casts of amorphous material, epithelial cells and sometimes leukocytes present near papillae. Many small venules contain fibrinous thrombi. Peripelvic tissues edematous. Pelves hemorrhagic, ulcerated and inflamed and contain desquamated tubular epithelium.
5	2.0	5	Diarrhea (1), anorexia (2), hematuria (3)	5	Gross: Kidneys normal in size but congested; pelves, left ureter and upper third of right ureter dilated; bladder markedly distended. On section, collecting tubules contain fine crystalline material. In both pelves are several blood-stained hard uroliths and bloody urine. Right pelvis also contains unorganized granular material. Upper third of right ureter dilated but not visibly obstructed. Left ureter obstructed in middle third by hard impacted mass of uroliths, above which are seven small calculi. Bladder inflamed, obstructed at urethral orifice and filled with bloody urine and many crystalline uroliths.
					Microscopic: There is advanced parenchymatous and fatty degeneration of tubular epithelium with contraction of lumens. Little or no fluid in capsular spaces. Glomeruli not compressed. Some collecting tubules slightly distended with fluid; others empty and contracted. Rarely there is ulceration, acute inflammation or early evidence of epithelial regeneration. No venous thrombi observed. Pelves contain blood and desquamated epithelial cells.
6	2.0	27	Hematuria (4), vomiting (5), anorexia (10)	28	Gross: Kidneys swollen; pelves dilated; no hydroureter; bladder contracted. On section, markedly dilated pelves contain masses of formed crystalline uroliths. Mucosal surfaces inflamed and hemorrhagic. Ureters and bladder grossly without lesion.
					Microscopic: Kidneys moderately congested. Marked parenchymatous degeneration of epithelium of convoluted tubules, which are distended with fluid and sometimes by hyaline casts. Fluid often extends into capsular spaces, distends these and compresses capillary tufts. Collecting tubules contracted and empty; some contain desquamated epithelial cells and rarely small masses of amorphous material, from which crystals appear to have been dissolved out. Tissues about these masses inflamed. Many venules contain fibrinous thrombi. Pelves moderately inflamed.

TABLE 1.—*Effect of Continued Oral Administration of Sulfapyridine to Monkeys—Continued*

Monkey	Dose (Gm. per Kg. per Day)	Duration of Medication (Days)	Symptoms and Day of Appearance	Day of Death	Pathologic Observations
7	4.0	4	Hematuria (3), complete anorexia (4)	5	Gross: Kidneys swollen and congested; pelves and ureters dilated; bladder contracted. On section, a small hard urolith is found embedded in renal papilla surrounded by hemorrhage. Other papillae hemorrhagic. Pelves and ureters contain bloody fluid, large quantities of crystalline and amorphous material and hard uroliths. Bladder inflamed and hemorrhagic and contains blood-stained urine, in which are needle-like crystals and a suspension of granular amorphous debris. Microscopic: Tubular epithelium shows parenchymatous and fatty degeneration. Glomeruli more cellular than normal. Collecting tubules contain fluid and occasional masses of a pale matrix, from which crystals appear to have been dissolved. Some tubules ulcerated and inflamed. Many venules contain fibrinous thrombi. Great numbers of epithelial cells, much blood and amorphous material present in pelves.
8	4.0	4	Vomiting (1), anorexia (2), hematuria (3)	5	Gross: Kidneys slightly edematous and markedly congested; pelves and right ureter dilated; left ureter dilated in upper third; bladder contracted. On section, pelves contain large quantities of blood and urine in which are suspended fine, needle-like crystals and amorphous granular debris. Right ureter obstructed at bladder and left in midportion by impacted hard uroliths. Bladder inflamed and contains extravasated blood and urine in which long, fine crystals and granular debris are suspended. Microscopic: Kidneys generally congested. Convoluted tubules generally contracted and empty. Epithelial cells show advanced parenchymatous and slight fatty degeneration. Cylinders of epithelium are separated from epithelium and in some cases invaginate capsular spaces, compressing glomerular tufts. Collecting tubules slightly distended by fluid, occasional red blood cells and small epithelial casts. Papillae focally hemorrhagic. Some venules contain fibrinous thrombi. Pelves inflamed and hemorrhagic, and contain fluid, desquamated epithelium and small numbers of red blood cells.

mals are still alive more than six months after the cessation of medication. During the interval they have acted as normal healthy animals, and have shown no objective sign of impaired function. The third animal of this group was put to death on the twenty-ninth day for the purpose of pathologic examination. Necropsy of this animal failed to reveal any gross lesions, but microscopic examination of various tissues indicated the presence of slight renal intratubular edema, slight congestion and small collections of amorphous debris at the papillae, which

TABLE 2.—*Effect of Continued Oral Administration of Sulfathiazole to Monkeys*

Monkey	Dose (Gm. per Kg. per Day)	Duration of Medication (Days)	Symptoms and Day of Appearance	Day of Death	Pathologic Observations
9	0.5	28	Diarrhea (1)	29 (killed)	Gross: Renal pelves, ureters and bladder grossly normal. Microscopic: Slight edema of convoluted tubules and congestion of glomeruli. Collecting tubules normal.
10	0.5	28	Diarrhea (1)	Alive and thriving six months after cessation of medication	
11	0.5	28	Diarrhea (12)	Alive and thriving six months after cessation of medication	
12	1.0	28	Nil	Alive and thriving six months after cessation of medication	
13	1.0	6	Anorexia (3), muscular weakness (4), hematuria (5)	8	Gross: Kidneys swollen and congested; bilateral hydropelvis and hydroureter; bladder moderately dilated. On section, kidneys appear acutely inflamed. Papillae hemorrhagic. Crystalline material present in tubules. Pelves hemorrhagic and, together with ureters and bladder, filled with soupy granular debris. Bladder also contains bloody urine and many discrete crystals. Microscopic: Epithelium of convoluted and collecting tubules shows marked parenchymatous and fatty degeneration. Many tubules contain acute purulent exudate, which often involves adjacent stroma and usually surrounds amorphous, castlike material, abundant in the larger collecting tubules. Necrosis, ulceration and desquamation of the epithelium sometimes present. Pelvic epithelium thickened; purulent exudate, erythrocytes and desquamated epithelial cells present.
14	1.0	4	Vomiting (1), anorexia (2), complete anorexia (4)	6	Gross: Kidneys swollen and congested; left hydroureter and hydropelvis; right normal; bladder contracted. On section, kidneys show foci of hemorrhage. Crystalline material present in larger collecting tubules. Dilated left pelvis filled with blood-stained granular debris and crystalline material. Right pelvis contains small quantity of purulent exudate. Blood-stained urine and large quantity of amorphous debris and crystalline material present in bladder. Microscopic: Many foci of acute inflammation in renal cortex together with focal necrosis of some tubules. Few similar foci in medullae centered about areas containing acellular, fibrilloid material. Marked parenchymatous and fatty degeneration of epithelium of convoluted and collecting tubules. Larger collecting tubules focally ulcerated and acutely inflamed, and contain accumulations of amorphous material. Mucosa of pelvis focally hemorrhagic and acutely inflamed.
15	2.0	4	Anorexia (3), monkey moribund (4)	5	Gross: Kidneys edematous and congested; bilateral hydropelvis and right hydroureter; bladder contracted. On section, papillae hemorrhagic and crystals in collecting tubules. Pelvis congested and contains bloody amorphous debris. Right ureter obstructed at bladder.

TABLE 2.—*Effect of Continued Oral Administration of Sulfathiazole to Monkeys—Continued*

Monkey	Dose (Gm. per Kg. per Day)	Duration of Medication (Days)	Symptoms and Day of Appearance	Day of Death	Pathologic Observations
					Microscopic: Kidneys edematous and congested. Moderate parenchymatous degeneration of tubular epithelium. Foci of acute inflammation distributed throughout cortex and medulla. Inflammatory foci surround pale, amorphous material containing empty spaces. Ulceration of epithelium of larger collecting tubules, which often contain epithelial casts. Mucosa of pelvis acutely inflamed.
16	2.0	24	Diarrhea (1), ataxia (7), anorexia (22)	24	Gross: Kidneys edematous; bilateral hydropelvis and hydroureter; bladder contracted. On section, marked congestion at papillae. Collecting tubules contain crystalline material. Pelves filled with blood-stained amorphous granular debris; mucosa hemorrhagic. Similar debris in ureters and bladder. Bladder acutely inflamed.
					Microscopic: Kidneys edematous. Varying degrees of parenchymatous degeneration of tubular epithelium, which is occasionally separated from basement membrane by fluid. Large collecting tubules contain pale-staining, mucoid-appearing material, and show ulceration, desquamation of epithelium and foci of acute inflammation. Pelvis shows area of acute inflammation, desquamation of epithelium and hemorrhage.
17	2.0	6	Cyanosis (4), ataxia (4), anorexia (5)	7	Gross: Kidneys edematous and congested; slight hydropelvis; no hydroureter; bladder contracted. On section, papillae hemorrhagic. Pelves contain blood-stained amorphous debris; mucosa inflamed. Bladder markedly congested and edematous.
					Microscopic: Kidneys congested and edematous. Moderate parenchymatous degeneration of convoluted tubules. Foci of necrosis and acute inflammation throughout cortex and medulla, chiefly involving tubules and adjacent stroma. Collecting tubules at papillae severely injured; epithelium ulcerated and desquamating; contain purulent casts, surrounded by inflammatory reaction. Mucosa of pelvis focally hemorrhagic and covered with acute inflammatory exudate.
18	4.0	5	Vomiting (4), hematuria (3)	6	Gross: Kidneys edematous; bilateral hydropelvis and right hydroureter; bladder contracted. On section, papillae hemorrhagic. Pelves contain blood-stained granular debris; pelvic mucosa hemorrhagic. Bladder edematous and inflamed.
					Microscopic: Marked intratubular edema. Numerous foci of acute inflammation and necrosis of tubular epithelium in cortex and medulla. Many large collecting tubules contain casts of finely granular amorphous material and sometimes show desquamation of epithelium and acute inflammation.

TABLE 2.—*Effect of Continued Oral Administration of Sulfathiazole to Monkeys—Continued*

Monkey	Dose (Gm. per Kg. per Day)	Duration of Medication (Days)	Symptoms and Day of Appearance	Day of Death	Pathologic Observations
19	4.0	4	Vomit (1), anorexia (2), hematuria (2)	4	Gross: Kidneys edematous and congested; bilateral hydropelvis and hydroureter; bladder contracted. On section, small hemorrhagic foci in dilated pelves. Small quantity of clear urine in bladder. Microscopic: Marked edema of tubules, sometimes of Bowman's capsules. Numerous foci of acute inflammation and necrosis, chiefly of tubules and adjacent stroma, in both cortex and medulla. Parenchymatous and fatty degeneration of convoluted tubular epithelium. Extreme changes in papillae; collecting tubules show ulceration and desquamation of epithelium, and contain purulent exudate and small castlike accumulations of amorphous fibrilloid material which often distends them. Epithelium of pelvis thickened. Mucosa focally hemorrhagic. Cellular debris, amorphous material, red blood cells and fluid present.
20	4.0	3	Hematuria (2), anorexia (2)	3	Gross: Kidneys edematous, slightly congested; hydropelvis and slight hydroureter; bladder contracted. On section, multiple petechial hemorrhages in pelvic mucosa. Granular debris present. Bladder congested, contains blood-stained urine and granular debris. Microscopic: Kidneys moderately congested and edematous. Epithelium often separated from basement membranes with compression of lumens; parenchymatous and fatty degeneration of epithelium marked. Foci of acute inflammation in some large tubules together with accumulations of amorphous debris and some desquamated epithelial cells.
21	5.0	3	Vomit (1), anorexia (2)	4	Gross: Right kidney edematous with small hemorrhage at lower pole; hydropelvis and hydroureter; left kidney normal size with bilobed pelvis and double ureter; bladder small. On section, right kidney congested; pelvis focally hemorrhagic; pelvis and ureter contain blood-stained urine and granular debris. Left kidney not remarkable on section; small amount blood-tinged debris in ureter. Bladder contains blood, urine and granular debris. Microscopic: Convoluted tubules edematous. Slight compression of glomerular tufts. Epithelium sometimes shows separation from basement membrane, probably by fluid. Collecting tubules contain casts of amorphous material, desquamated epithelial cells, erythrocytes and occasional neutrophils.
22	5.0	3	Anorexia (3)	4	Gross: Kidneys edematous and congested; bilateral hydropelvis and left hydroureter; bladder contracted. On section, pelves contain bloody granular debris. Bloody granular material containing many fine crystals, some sticking into vesical mucosa, is present. Mucosa markedly congested. Microscopic: Parenchymatous and fatty degeneration of epithelium of convoluted tubules. Minute foci of acute inflammation sometimes present. Some collecting tubules contain hyaline casts.

TABLE 2.—*Effect of Continued Oral Administration of Sulfathiazole to Monkeys—Continued*

Monkey	Dose (Gm. per Kg. per Day)	Duration of Medication (Days)	Symptoms and Day of Appearance	Day of Death	Pathologic Observations
23	10.0	8	Anorexia (6), ataxia (7)	9	Gross: Kidneys pale; slight hydropelvis and hydroureter; bladder contracted. On section, minute cortical hemorrhages. Crystals in collecting tubules at papillae. Mucosal surface of pelvis hemorrhagic. Pelves and ureters contain blood-stained amorphous debris and abundant crystals. Bladder mucosa congested. Blood-stained amorphous debris with crystals and crystalline calculi present. Microscopic: Kidneys edematous. Extremely marked fatty and parenchymatous degeneration of convoluted tubular epithelium. Epithelium of collecting tubules degenerated and desquamated with active regeneration. Neutrophils and macrophages in capillaries of many glomeruli. Large collecting tubules distended with amorphous and hyaline casts mixed with epithelial cells, neutrophils and erythrocytes. Desquamated cells and granular and hyaline debris in pelvis. Pelvic mucosa hemorrhagic.
24	10.0	5	Hematuria (4), anorexia (4)	5	Gross: Kidneys externally normal; hydropelvis and hydroureter; bladder contracted. On section, papillae of left side congested, where crystalline material is present in collecting tubules. Pelves and ureter congested and contain masses of crystals. Vesical mucosa focally congested; blood-tinged urine and crystalline masses present. Microscopic: Kidneys moderately congested and edematous. Extreme degree of parenchymatous and fatty degeneration of the epithelium of the convoluted tubules and swelling, disintegration and desquamation of the epithelium of collecting tubules. Focal necrosis of tubular epithelium marked. Many collecting tubules contain hyaline and amorphous casts and purulent exudate. Regeneration of epithelium is marked.

TABLE 3.—*Average Blood Concentrations of the Drugs in Monkeys During the Administration of 0.5 Gm. per Kilogram per Day*

Drug	Free	Total
Sulfapyridine	5.3	12.8
Sulfathiazole	4.0	4.3

likewise showed a low grade inflammatory reaction. The pyelitis was not of the same order of severity as that seen in the sulfapyridine series.

Figure 3 shows the blood concentrations of the drug resulting from the administration of this dose. On casual examination it appears that the administration of 0.5 Gm. per kilogram per day of sulfapyridine

produces a considerably higher level than does the administration of an equivalent dose of sulfathiazole. If averages are made and the terminal peak concentration which is associated with the onset of fatal toxic

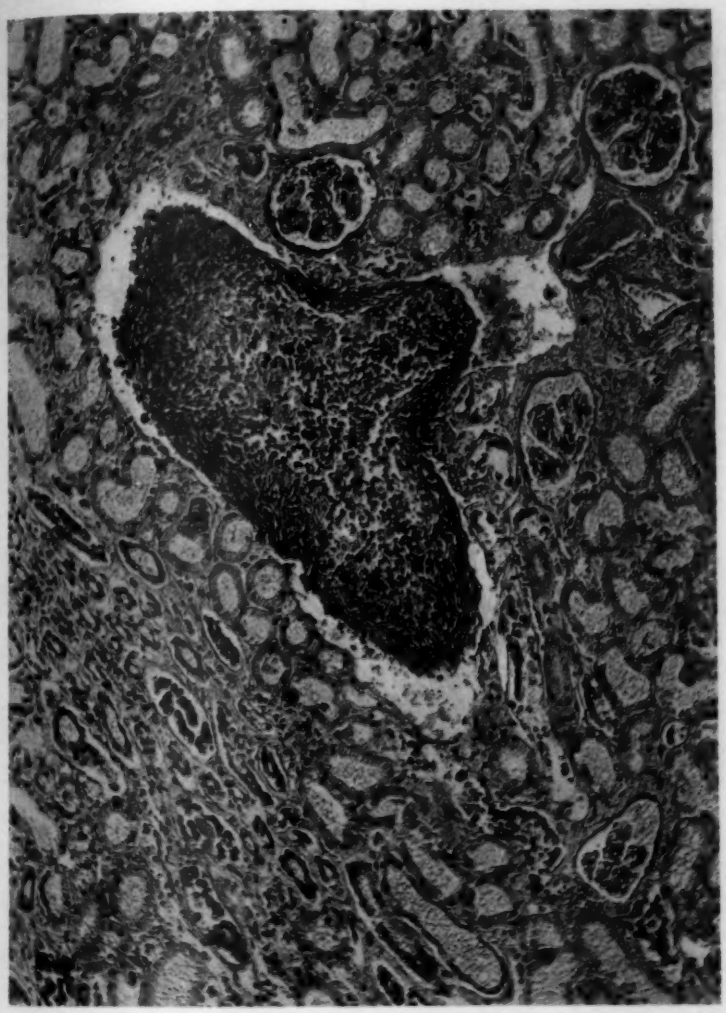


Fig. 1.—Kidney from monkey 26. This animal was given sulfapyridine, 0.5 Gm. per kilogram per day. Death occurred on the twenty-fifth day. A typical acute fibrinous thrombus in a branching venule of the kidney is seen. Note the marked edema of convoluted tubules and glomerular spaces and the degenerative changes in the collecting tubules at the right. × 110.

symptoms is disregarded, one finds that this difference is almost entirely dependent on the amount of the conjugated drug present.

Thus, while there is a threefold difference between sulfathiazole and sulfapyridine as regards the total concentration of the drug in the blood, the difference as regards the unconjugated, or therapeutically effective, form of the drug is only about 20 per cent.

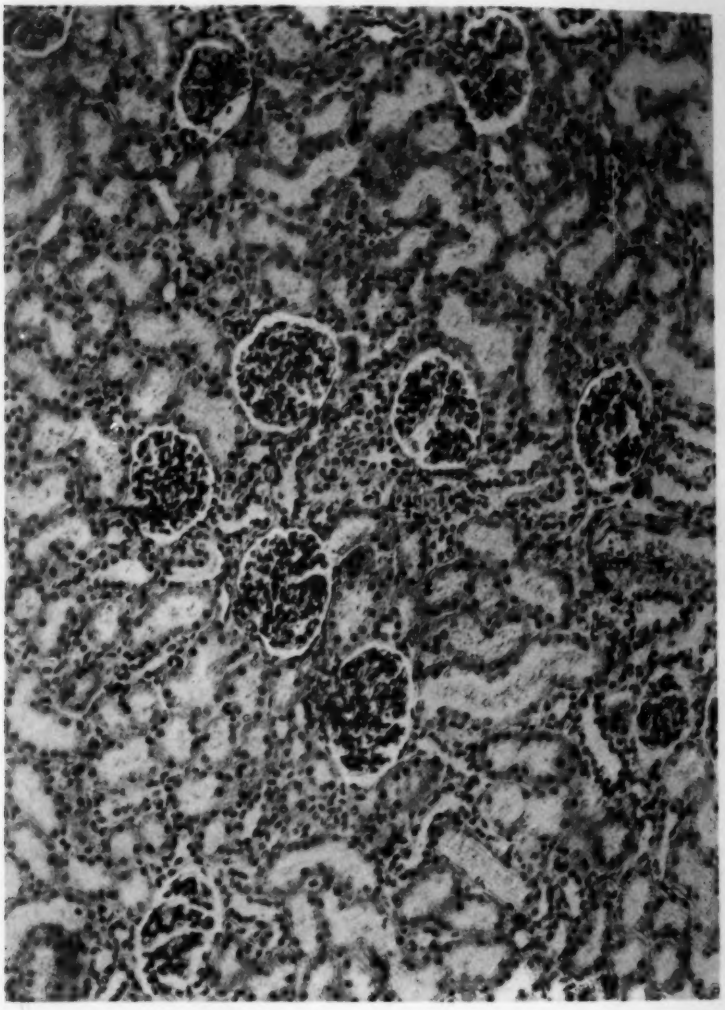


Fig. 2.—Kidney from monkey 9. This animal was given sulfathiazole, 0.5 Gm. per kilogram per day, and was killed on the twenty-ninth day. Aside from slight congestion and moderate edema of the convoluted tubules, no changes of note are present. × 150.

When the dose level is increased to 1.0 Gm. per kilogram per day, the striking difference between sulfapyridine and sulfathiazole as

regards toxicity tends to disappear. At this level, 2 of a group of 3 animals on sulfapyridine died on the seventh day of medication, while the remaining animal survived twenty-eight days of medication but died on the thirtieth day or two days after the administration of the drug had been stopped. In the sulfathiazole series 2 animals died on the sixth and eighth days of the experiment. One animal survived twenty-eight days of medication, during which time no untoward reactions were observed. This animal is still alive and healthy more than six months after completion of the experiment.

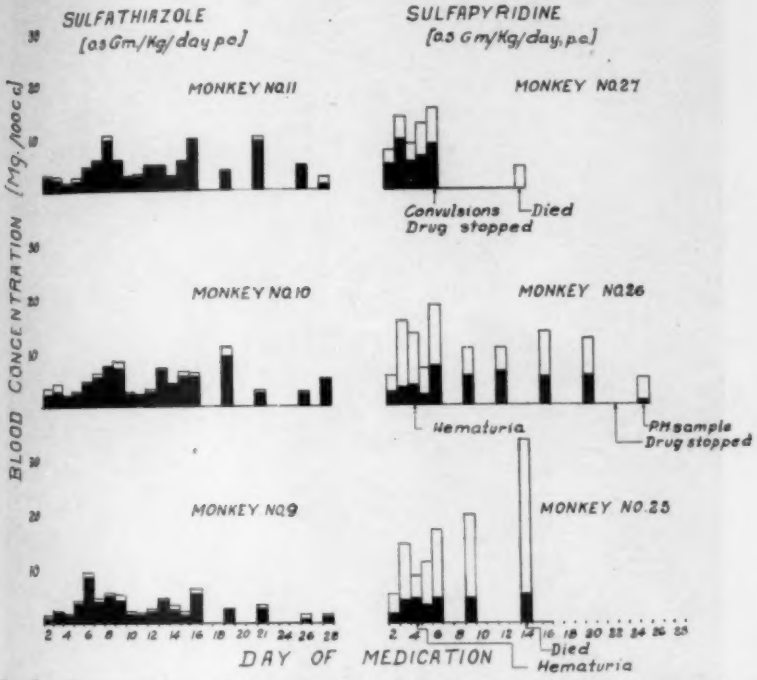


Fig. 3.—Concentrations of drugs in blood of monkeys given 0.5 Gm. per kilogram per day.

In 2 of the 3 animals on sulfapyridine frank hematuria developed together with severe anorexia and a correspondingly marked loss of weight. The primary lesions noted were resultant to urolithiasis. The kidneys were markedly edematous; there were bilateral hydronephrosis and hydroureters. The congested papillae contained crystalline material.

The dilated renal pelves were invariably the site of an acute inflammatory reaction associated with numerous submucosal petechial hemorrhages. Formed uroliths, from 1 to 5 mm. in diameter, were present. The ureters were dilated throughout their entire course and were often obstructed at the ureterovesical junction by an impacted mass of blood-

stained crystalline and granular debris which formed a cast of the ureter. The bladder itself was the site of an acute inflammatory reaction centering about the papillae and involving the trigone as well.

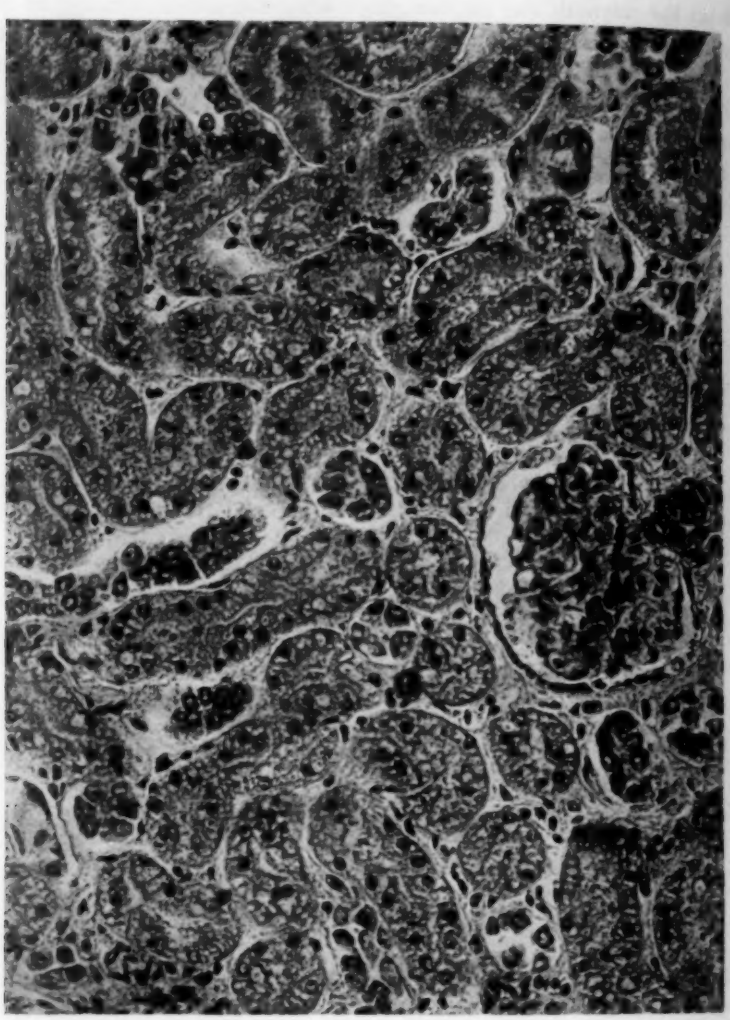


Fig. 4.—Kidney from monkey 3. This animal was given sulfapyridine, 1 Gm. per kilogram per day. Death occurred on the eighth day. Extreme fatty degeneration of collecting tubules is present. The changes are so marked that the condition is practically severe nephrosis. × 240.

Microscopically, these kidneys showed generalized edema and slight congestion. There was a marked increase in the size of the capsular spaces, which were filled with an albuminous material. In spite of

moderate congestion throughout the entire kidney, the tufts of the glomeruli were constricted and empty, occupying only a small portion of the capsule. Both the convoluted tubules and the collecting tubules

Fig. 5.—Kidney from monkey 13. This animal was given sulfathiazole, 1 Gm. per kilogram per day. Death occurred on the eighth day. Precipitated drug in a collecting tubule is seen. Note that epithelium is missing and that acute inflammatory reaction is pronounced. × 240.

were markedly dilated. The epithelium of the convoluted tubules showed a generalized, diffused parenchymatous and fatty degenerative change

of a severe degree, while the epithelial cells of the collecting tubules showed fatty degeneration with focal areas of necrosis and desquamation of the epithelial elements into the lumens of the tubules. In a number

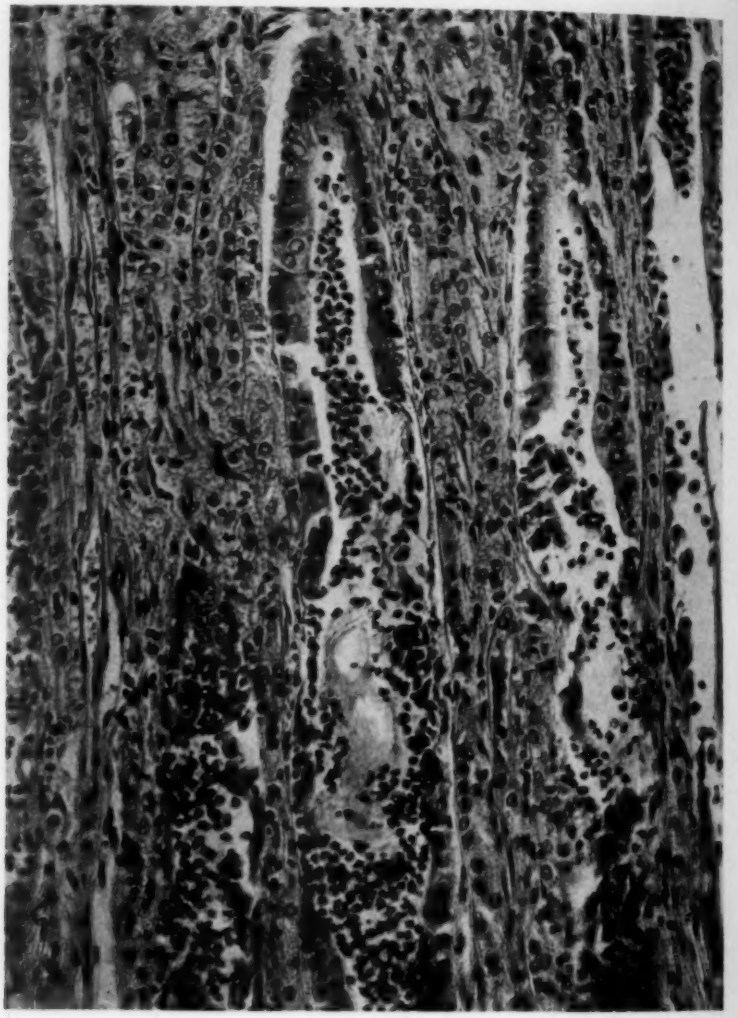


Fig. 6.—Kidney from monkey 13. This animal was given sulfathiazole, 1 Gm. per kilogram per day. Death occurred on the eighth day. Collecting tubules containing crystals and acute inflammatory exudate are shown. Note crystals of drug impinging on and injuring epithelium with production of minute ulcerations. × 230.

of instances, hemorrhagic casts were seen. Numerous thrombosed venules were distributed throughout the cortex and medulla. The lesions at the

renal papillae and the pelvis were severe. The area showed an acute inflammatory reaction with a diffuse infiltration of neutrophilic leukocytes and desquamation of overlying epithelium. In addition to the renal

TABLE 4.—*Average Blood Concentrations of the Drugs in Monkeys During the Administration of 1.0 Gm. per Kilogram per Day*

Drug	Free	Total
Sulfapyridine	5.6	18.9
Sulfathiazole	10.1	15.9

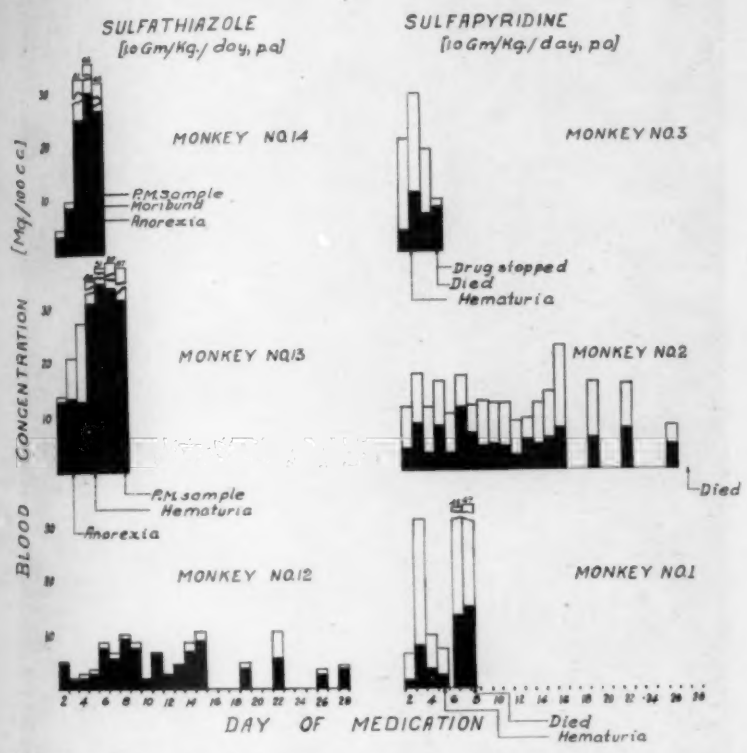


Fig. 7.—Concentrations of drugs in blood of monkeys given 1 Gm. per kilogram per day.

lesion, slight general parenchymatous degeneration was found in the liver, while the spleen showed lymphoid hyperplasia and neutrophilia of the splenic pulp, indicative of toxic splenitis, and moderate erythrophagocytosis.

Macroscopically, the lesions in the renal tracts of the animals on an equivalent dose of sulfathiazole (1.0 Gm. per kilogram per day) appeared to be less severe than those observed in the sulfapyridine

series. This was primarily due to the fact that no gross obstructions were present and that no formed calculi were observed. The kidneys were edematous and congested, and the cut surface had a peculiar stri-

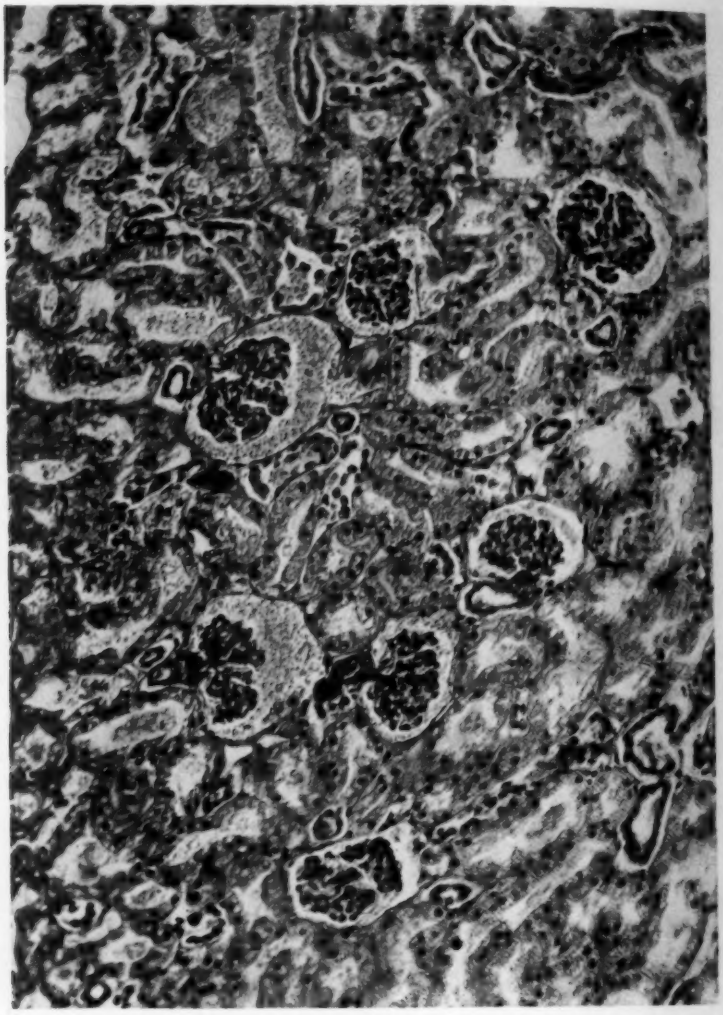


Fig. 8.—Kidney from monkey 4. This animal was given sulfapyridine, 2 Gm. per kilogram per day. Death occurred on the seventh day. Note the edema of the tubules and especially of the glomerular capsules. The capillary tufts appear compressed. × 150.

ated appearance, which seemed to accentuate the normal markings. The papillae were hemorrhagic, and the dilated pelves contained a large

amount of amorphous blood-stained sandlike material, in which discrete crystalline bodies could be observed. Crystalline material was also visible in the collecting tubules. Granular material and blood-stained

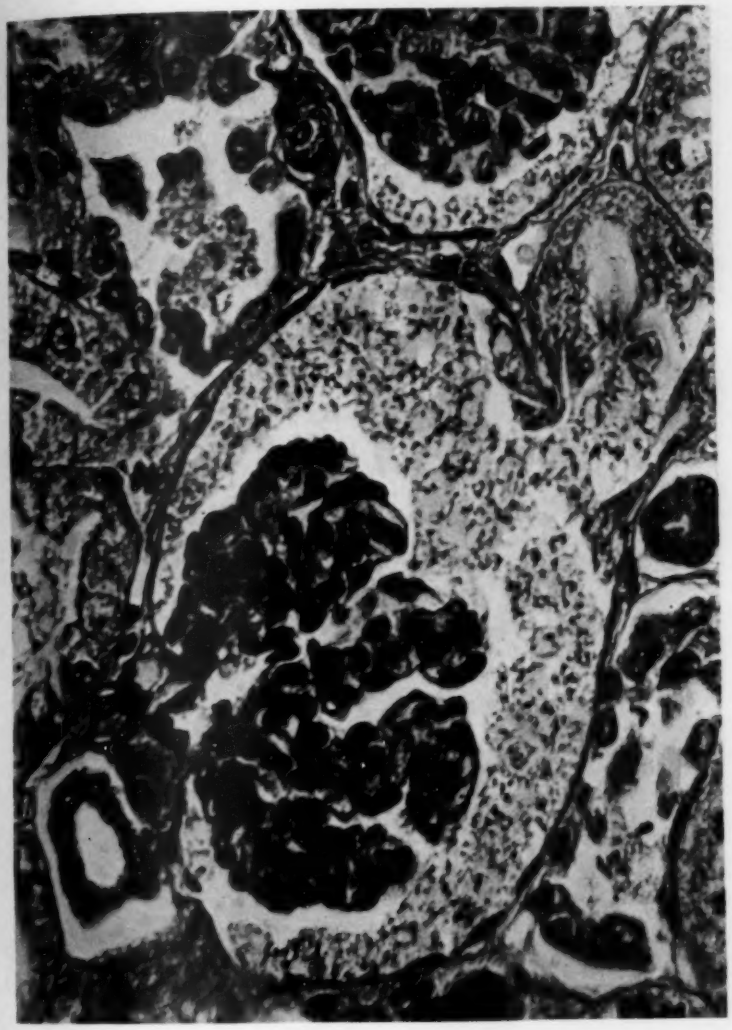


Fig. 9.—High power photomicrograph of a glomerulus in kidney from monkey 4, which received sulfapyridine in the dosage of 2 Gm. per kilogram per day. Note the marked edema and compression of the capillary tuft. × 600.

debris were also present in the bladder. Chemical analysis of this material showed it to contain both free and acetylated sulfathiazole, the latter form predominating.

Microscopically, the lesions in this series were more severe than those in the analogous sulfapyridine series, giving the impression of acute pyelonephritis with multiple foci of acute inflammation distributed

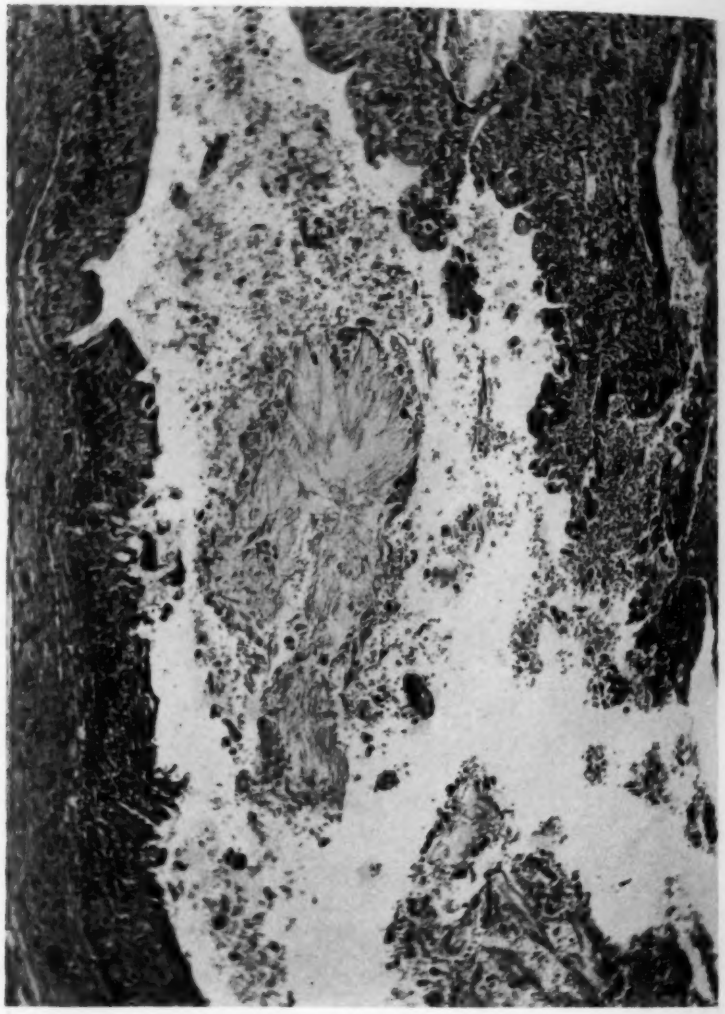


Fig. 10.—Kidney from monkey 7. This animal was given sulfapyridine, 4 Gm. per kilogram per day. Death occurred on the fifth day. The pelvis shows acute ulceration of its lining epithelium and contains crystalline material, amorphous debris and epithelial and blood cells. × 100.

throughout the cortex and medulla. These proved to be centers of acute irritation with necrosis of the epithelial elements of the tubule,

surrounded by an intense neutrophilic reaction, which often extended into the adjacent interstitial tissues. The acellular central zone of such an area invariably contained a number of clear slitlike spaces that suggested something had been removed. It is probable that crystals of sulfathiazole, or acetylated sulfathiazole, had been present in those central spaces and were later dissolved out by the histologic reagents. In

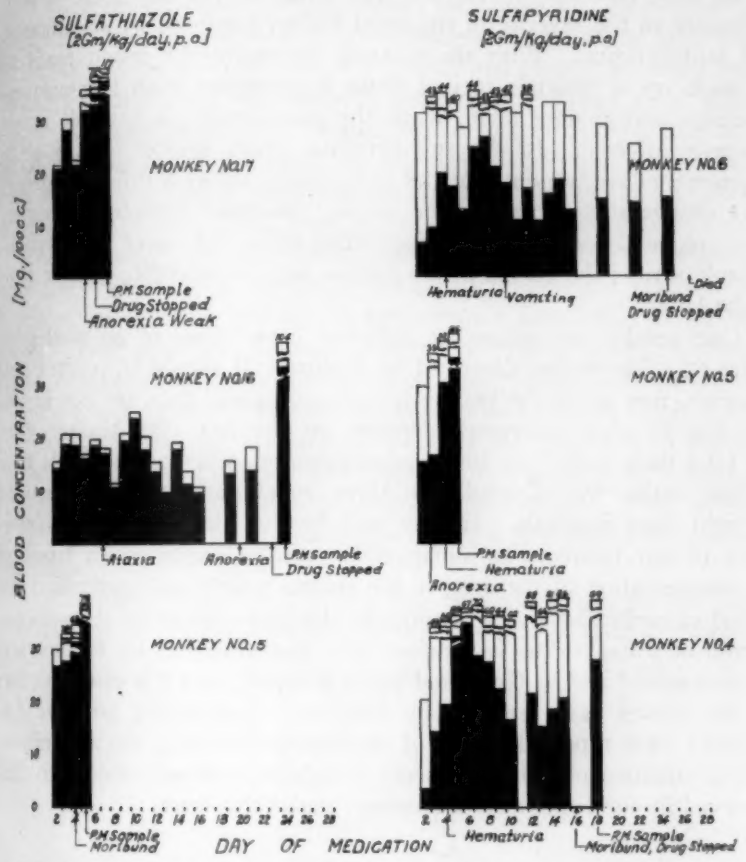


Fig. 11.—Concentrations of drugs in blood of monkeys given 2 Gm. per kilogram per day.

l instance frozen sections of the kidney were treated with the reagents used for quantitative demonstration of sulfathiazole. It was possible to demonstrate the presence of the drug in the larger collecting tubules by this method. It may be inferred that these crystalline deposits were the irritants which evoked the acute inflammatory reactions.

At this dose level the difference in blood concentration in the sulfathiazole and sulfapyridine series becomes less apparent.

Here again, the difference in the rate and degree of conjugation is clearly manifested; while the total concentration of sulfapyridine is slightly higher than that of sulfathiazole, the concentration of free sulfathiazole is almost twice the concentration of free sulfapyridine. Figure 7 shows the daily blood concentrations of the drugs for all animals at this dose level.

At dose levels above 1.0 Gm. per kilogram per day there is little difference in the severity of the renal lesions produced by sulfathiazole and sulfapyridine. With the thiazole derivative the lesion tends to be made up of multiple foci of acute degeneration with inflammatory reactions surrounding them, while the parenchyma as a whole is not seriously affected. In the sulfapyridine series, similar foci of acute degeneration are present, but the parenchyma shows a diffuse degenerative change suggestive of toxic action. Multiple thrombosed venules were frequently seen in the sulfapyridine series but never in the sulfathiazole series; the edema in the former was considerably greater than in the latter.

Our results are somewhat different from those of an analogous series on sulfapyridine described by Molitor. It should be pointed out, however, that while the two series are analogous, they are not identical; the principal difference depends on the fact that Molitor gave the total daily dose in a single administration, whereas the day's dose in this series was divided into three equal parts and administered at eight hour intervals. It may well be that the increased susceptibility of our monkeys to sulfapyridine can be explained in terms of the concentration of the drug in the tissues maintained throughout the period of medication. The pathologic picture produced by the administration of sulfapyridine is identical with that described by Molitor and his colleagues [6] and by Gross and his co-workers,[7] and it is similar to that in the human case reported by Stryker.[8] Lowenburg and his colleagues [9] have reported a case of urolithiasis following the administration of sulfathiazole which presents a pathologic picture similar to that observed in our experimental monkeys under this drug.

SUMMARY

Sulfathiazole and sulfapyridine in doses from 0.5 Gm. per kilogram per day up to 10 Gm. per kilogram per day were administered to monkeys for a maximal period of twenty-eight days in order to obtain some evi-

6. Antopol, W., and Robinson, H.: Proc. Soc. Exper. Biol. & Med. **40**:428, 1939; Arch. Path. **29**:67, 1940.

7. Gross, P.; Cooper, F. B., and Lewis, M.: Urol. & Cutan. Rev. **43**:299, 1939.

8. Stryker, W. A.: J. A. M. A. **114**:953, 1940.

9. Lowenburg, S. A.; Sloan, N. G., and Chodoff, P.: J. A. M. A. **114**:2069, 1940.

dence of the comparative toxicity of these two compounds. The drugs were administered by stomach tube as milk suspensions at eight hour intervals throughout the entire period of medication. Daily observations of blood concentrations were made.

At a dose level of 0.5 Gm. per kilogram per day animals given sulfapyridine died on the thirteenth, fourteenth and twenty-fourth days of medication, respectively. Hematuria was present in all. At postmortem examination, urolithiasis, degenerative changes of the tubular epithelium, particularly of the collecting tubules, pyelitis and cystitis were observed. Monkeys receiving the same dose of sulfathiazole showed no ill effects during the twenty-eight days of medication. One animal of the latter series, killed on the twenty-ninth day for necropsy, showed no significant pathologic changes other than slight edema of the kidney and a chronic inflammatory process of the renal pelvis.

The difference between sulfathiazole and sulfapyridine disappeared when the dose level was raised; at and above 1.0 Gm. per kilogram per day fatalities occurred and severe renal lesions were observed in both series. These manifested themselves as parenchymatous and fatty degenerative changes of the epithelium of the collecting tubules, associated with the presence of crystalline material, focal necrosis and focal inflammation, and ulceration, necrosis and desquamation of the epithelial elements of the larger collecting tubules. The renal pelves showed acute inflammatory reactions associated with submucosal hemorrhages. The severity of the lesions varied directly with the height and duration of the concentration of the drug in the blood.

It should be pointed out that the dose range employed in this series approximates ten to two hundred times the usual therapeutic range.

Case Reports

STIMULATION OF GONADS ASSOCIATED WITH HYPERINSULINISM IN AN INFANT

MIRIAM C. BENNER, M.D., DENVER

It is well known that the use of insulin has increased the fertility of diabetic women, has made possible the survival of diabetic girls to the child-bearing age and has decreased the maternal mortality rate during, and immediately after, pregnancy. It is a noteworthy fact, however, that fetal mortality has not been altered significantly by the treatment of diabetes in the mother, as infants born to such women are frequently stillborn or die soon after birth. A number of investigators have suggested that this high infant mortality is due to fetal compensation for the diabetes of the mother and that after birth, when compensation is no longer necessary, hypoglycemia may develop in the infant to the point of causing death. Evidence for this opinion has been obtained from the observations that in some cases the disease of the mother is less severe during the latter half of pregnancy, that the infant's blood sugar levels are often low and that hyperplasia and hypertrophy of the islands of Langerhans have been reported in a number of infants who have died soon after birth. There is considerable doubt, however, that fetal and neonatal deaths of infants of diabetic mothers can be attributed to overactivity of the fetal islet tissue in all, or even the majority, of cases, since (*a*) remission of the maternal disease during late pregnancy is by no means a universal finding and many women show an exacerbation of their diabetes, since (*b*) Hartmann [1] has reported as low blood sugar levels (20 to 40 mg. per hundred cubic centimeters) in infants born to nondiabetic women as in those born to diabetic women and since (*c*) morphologic evidence of hypertrophy and hyperplasia of the islands of Langerhans is found in only a limited number of the infants who come to autopsy. Those cases in which an excessive amount of islet tissue is present cannot be disregarded, however, and serve to show that in some instances at least the death of the infant may be the result of overactivity of the islet tissue.

Recent studies by White, Titus, Joslin and Hunt [2] serve to illustrate the complexity of the problem. These workers followed pregnancy in many diabetic women in whom the disease was well controlled and noted that the fetal mortality was not much affected by careful management of the maternal diabetes. They found that stillbirth was antedated by toxemia and that the toxemia was not related to the diabetes but rather to a hormonal imbalance, which was shown by an

From the Child Research Council and the Department of Pathology, University of Colorado School of Medicine.

1. Hartmann, A. F.: J. Iowa M. Soc. **28**:1, 1938.

2. White, P.; Titus, R. S.; Joslin, E. P., and Hunt, H.: Am. J. M. Sc. **198**:482, 1939.

increase in the level of gonadotropin in the blood. Furthermore, treatment directed toward reduction of this substance appeared to bring about a disappearance of toxic symptoms and subsequent delivery of a "normal" infant at term. If the conclusions of these workers are correct, it would be logical to expect that there might be morphologic evidence in the fetus of the effects of the increase in gonadotropin. A case reported by Smyth and Olney [3] is suggestive, as there were not only marked hypertrophy and hyperplasia of the islands of Langerhans but evidences of stimulation of follicles and luteinization in the ovaries, as well as changes in the uterus suggestive of early menstruation.

An infant was seen at the Colorado General Hospital which showed changes consistent with an endocrine imbalance involving the islands of Langerhans and also the gonads. The case of this infant will be reported at this time in order that it may be added to the material being accumulated on the subject of the endocrine abnormalities observed in connection with diabetes in pregnancy.

REPORT OF A CASE

A 35 year old multipara entered the clinic in 1931, giving a history of having had a girl weighing 10½ pounds (4,762.5 Gm.) in 1916, a boy weighing 11½ pounds (5,216 Gm.) in 1928 and a miscarriage in 1930. Her blood sugar was found to be increased to 228 mg. per hundred cubic centimeters. She was placed on a diabetic diet, which reduced the blood sugar to normal. This patient was uncooperative and reported at the clinic only at rare intervals; she refused to take insulin at any time. In 1936 she returned to the clinic and stated that her urine had not been tested for sugar for about five years and that she had been pregnant five months. Urinalysis showed no sugar, and the blood was not examined. March 10, 1937 she entered the hospital in labor; her blood pressure was 148 systolic and 90 diastolic and hydramnios was present. The patient stated that the membranes had ruptured three days previously and that a small amount of fluid had been discharged. She was delivered of a girl, weighing 10¼ pounds (4,449 Gm.), on March 11. After delivery her urine was negative for sugar; the blood was not examined. The patient was dismissed from the hospital to be followed in the clinic but has not returned.

The infant was in poor condition at birth, and a Flagg respirator was used to induce breathing. She had frequent spells of severe cyanosis, during which she appeared to stop breathing for short intervals. Oxygen was given, with temporary relief of symptoms. Eight hours after birth 150 cc. of 5 per cent dextrose was given subcutaneously, and three hours later 15 cc. of whole blood was injected into the buttock. These procedures did not bring about any lasting improvement in the condition of the infant, and she died twenty-two hours after birth.

At autopsy the body was that of a well nourished, well developed white girl weighing 4,536 Gm. and measuring 55 cm. in length. Cyanosis of the skin was extreme. The positive gross findings included cardiac enlargement (35 Gm.), congestion of the lungs, petechial hemorrhages in the pleura, thymus, epicardium and meninges, moderate enlargement of the liver (205 Gm.), small subcapsular hemorrhages in that organ and uric acid infarcts in the kidneys. The pancreas weighed 4 Gm. and was a light purple-gray color. The uterus was normal in size and

3. Smyth, F. S., and Olney, M. B.: J. Pediat. **13**:772, 1938.

was severely congested; its mucosa appeared hemorrhagic. The fallopian tubes were swollen and congested. The ovaries were enlarged, measuring 2.5 by 1.5 by 0.5 cm., and contained many cysts.

Microscopic examination showed the following changes: The lungs were congested, and the alveoli contained solid elements from the amniotic fluid; there

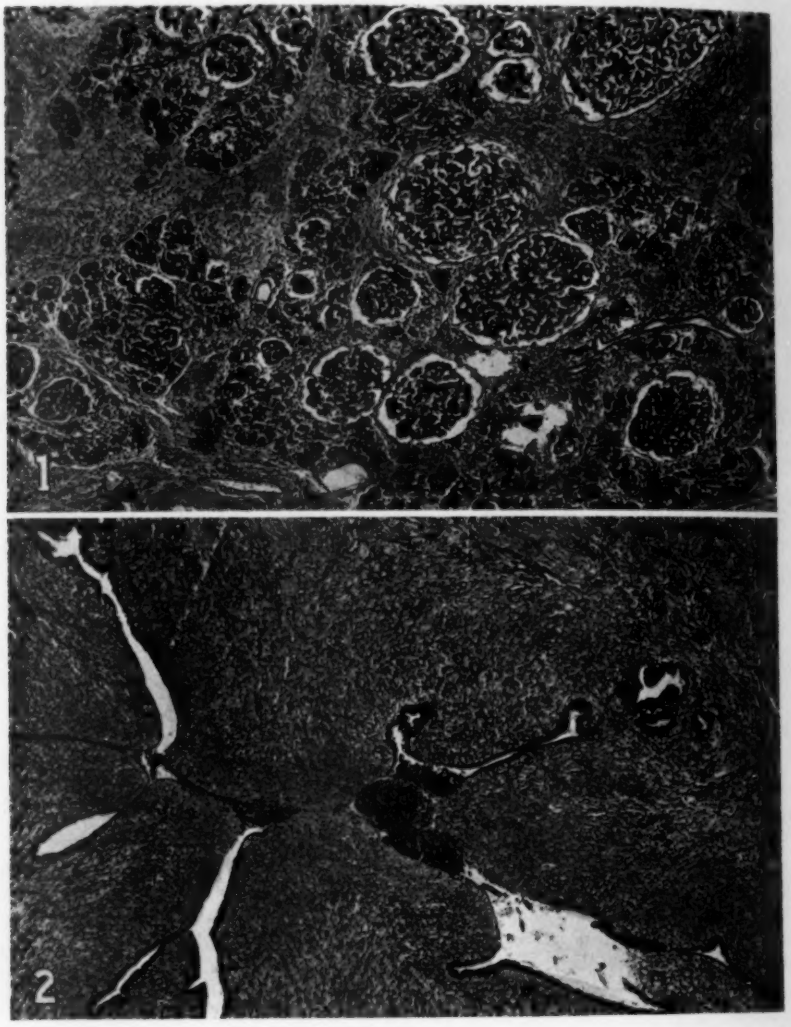


Fig. 1.—Photomicrograph (× 50) of a section of pancreas showing hyperplasia and hypertrophy of the islands of Langerhans.

Fig. 2.—Photomicrograph (× 50) of a section of uterus showing hemorrhage into the endometrium.

was no evidence of inflammation. The liver was congested and showed moderate fatty metamorphosis and many foci of hemopoiesis. The adrenals contained a few

small areas of focal necrosis with polymorphonuclear infiltration in the fetal cortex. In the pancreas there was a tremendous increase in both the number and the size of the islands of Langerhans; individual islands contained many large hyperchromatic cells. The pancreatic acinous tissue was relatively decreased in amount,

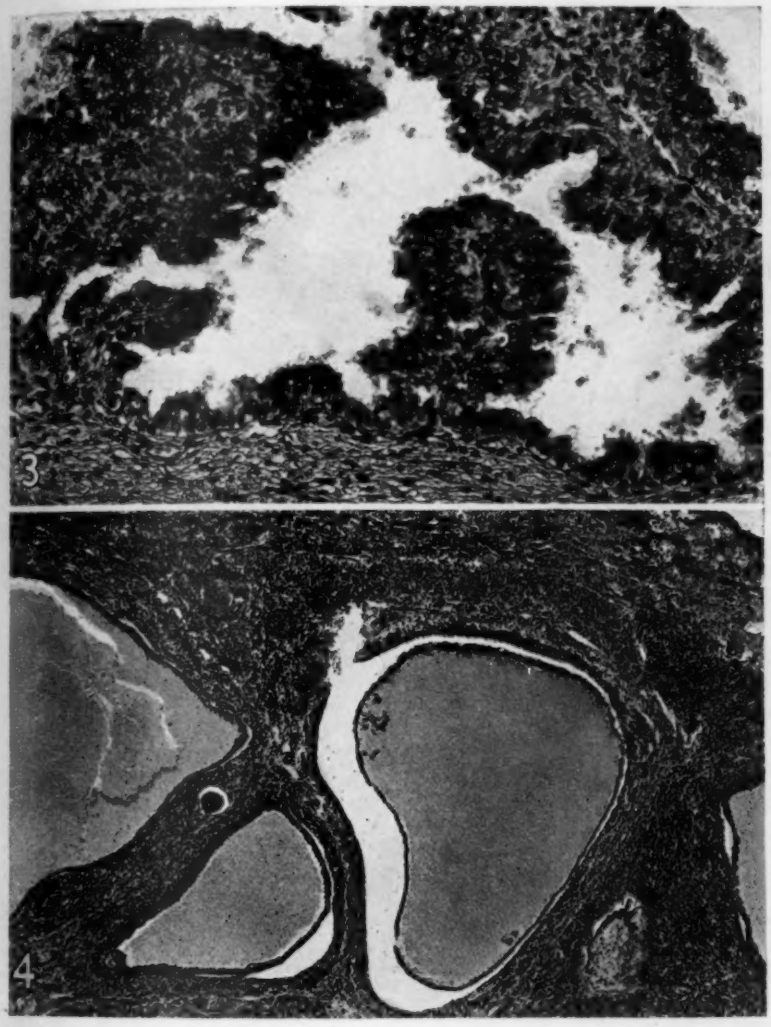


Fig. 3.—Photomicrograph (× 175) of a section of fallopian tube showing epithelial hyperplasia and leukocytic infiltration into the stroma.

Fig. 4.—Photomicrograph (× 36) of a section of ovary showing follicular cysts and lutein reaction.

and both the stroma and the parenchyma were diffusely infiltrated with neutrophils, eosinophils and lymphocytes (fig. 1).

The internal genitalia were quite unusual in appearance. In the uterus there was extravasation of red blood cells into the endometrium and also slight desquamation of the surface epithelium; some of the glands were very slightly branched, and the endometrium was well defined (fig. 2). The fallopian tubes showed large

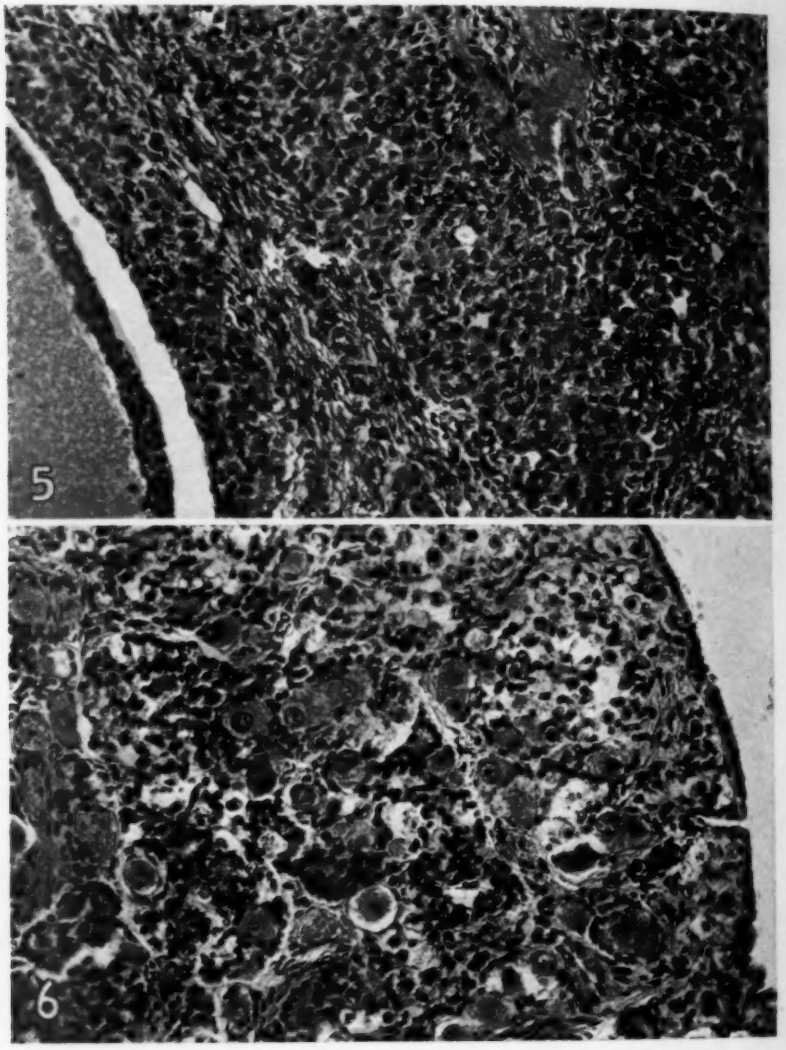


Fig. 5.—Photomicrograph (× 175) of a section of a portion of the ovary shown in figure 4.

Fig. 6.—Photomicrograph (× 175) of a section of a normal ovary from a newborn infant.

numbers of polymorphonuclear neutrophils in the stroma of the villi and a few in the lumen. The tubal epithelium was made up of a heavy layer of pseudo-

stratified columnar epithelium in which the cells were closely crowded together (fig. 3). The ovaries (figs. 4 and 5) showed a remarkable deviation from the usual morphologic picture (fig. 6); they contained many cysts measuring up to about 1 cm. in diameter filled with pink-staining precipitate and lined by a granulosa cell layer two or three cells in thickness. In two of the cysts scattered polymorphonuclear leukocytes lay adjacent to the granulosa cells. Surrounding many of the follicular cysts and also throughout the stroma were many large, slightly foamy polygonal cells which were histologically typical of theca lutein cells; these cells made up a large part of the ovary, but no definite circumscribed corpus luteum was found. The primordial follicles appeared to be somewhat reduced in number, but this may have been due to the marked increase in the size of the ovary and may therefore have been relative.

The breast tissue measured about 5 mm. in greatest diameter and consisted of ducts packed closely together in a scanty connective tissue stroma and moderately infiltrated with lymphocytes.

COMMENT

This woman did not complain of toxic symptoms at any time during her pregnancy, but at the time of delivery she had a definite hydramnios and a slight elevation of blood pressure. She was known to be extremely unconcerned about her diabetes and apparently made no effort to limit her diet or to have her urine examined for sugar. These facts lead me to believe that she suffered from both unregulated diabetes and some degree of toxemia.

The infant was large and was never in good condition. Injections of dextrose did not appear to bring about any significant improvement in the child's condition, but it may be that this therapy should have been continued, especially since at autopsy the pancreas showed such marked hypertrophy and hyperplasia of the islet tissue. It is probable that the death of the baby was due to hyperinsulinism, although the aspiration of amniotic fluid and the hemorrhages in various organs certainly decreased her chance of survival.

The unusual findings in the genitalia tend to support the hypothesis of White and co-workers [2] that there is a marked endocrine imbalance in some pregnant diabetic women which may affect the infant. The changes observed in the case presented indicate both follicle stimulation and a luteinizing effect on the stromal, or thecal, cells. The hyperplasia of the epithelium of the fallopian tubes also suggests abnormal endocrine stimulation, as does the hemorrhage into the endometrium of the uterus. It is difficult to evaluate the leukocytic infiltrations seen in the pancreas, ovaries, fallopian tubes, breast and, to a lesser degree, in the uterus. Such infiltrations have been reported frequently in connection with hyperplastic islet tissue in the pancreas and also in connection with hormone stimulation of the genitalia. It seems probable, therefore, that the presence of the leukocytes does not indicate an inflammatory process but, rather, an endocrine abnormality. Confirmation of this opinion was obtained from the fact that the thymus gland did not show the involutionary changes usually seen in infectious disease in infants.

The evidence presented in this case indicates that there may be definite gonadotropic stimulation in connection with hyperfunction of the insulin apparatus in some infants born to diabetic mothers but does

not permit any conclusions as to the source of the follicle-stimulating or luteinizing factors. Chorionic gonadotropin is known to be capable of inducing such stimulation, but the pituitary gland may produce similar changes in animals,[4] and it is well known that this gland may be primarily involved in diabetes. Therefore, one must consider the possibility that the ovarian changes which have been observed may be a reflection of abnormal function of the pituitary gland rather than of the chorionic tissue. The fact that Liegner [5] and Crainicianu and Copelman [6] found that injections of insulin appeared to stimulate the ovaries in sterile nondiabetic women must also be taken into account as indicating the possibility that hyperinsulinism itself may be capable either of acting directly on the ovaries or of modifying ovarian function through the pituitary gland.

SUMMARY

A case has been presented in which there is morphologic evidence of gonadotropic stimulation as well as hyperfunction of the islands of Langerhans in an infant born to a diabetic woman. The evidence presented is not sufficient to indicate whether the follicle stimulation and luteinization observed in the ovaries are a reflection of the presence of an excess of chorionic gonadotropin, of abnormal pituitary function or of hyperinsulinism.

4. Engle, E. T., and Levin, L.: J. A. M. A. **116**:47, 1941. Smith, P. E.: ibid. **115**:1991, 1940.

5. Liegner, B.: Zentralbl. f. Gynäk. **58**:2952, 1934.

6. Crainicianu, A., and Copelman, L.: Compt. rend. Soc. de biol. **121**:1303, 1936.

Laboratory Methods and Technical Notes

A NEW STAINING METHOD FOR GRAM-POSITIVE AND GRAM-NEGATIVE ORGANISMS IN FROZEN SECTIONS

ARAM A. KRAJIAN, LOS ANGELES

The importance of a staining method for the demonstration of gram-positive and gram-negative organisms in tissue sections cannot be overemphasized. One is often called on to employ a stain as an aid in establishing a rapid correct diagnosis of a condition presented in biopsy and autopsy material.

Christian Gram [1] in 1884 devised his method for the demonstration of bacteria in tissue sections. Weigert's [2] modification of Gram's method has been useful for the demonstration of fibrin and bacteria and is still widely used, but both methods demonstrate only gram-positive organisms.

Since then numerous attempts have been made to demonstrate both gram-positive and gram-negative organisms in paraffin sections of tissues that had been fixed in Zenker's solution or in formaldehyde solution, and methods accomplishing this purpose have been reported by MacCallum,[3] Lyon,[4] Lillie,[5] Brown and Brenn,[6] Rudnikoff and Stawsky,[7] Glynn [8] and others.

In these modifications the staining solutions as devised by Gram remained practically unchanged. No attempt has been made to devise a new method by the use of some other dye or chemical, and no recommendations have been made as to the applicability to frozen sections.

The solutions used by Gram and used in the modifications of his method are: gentian violet, methyl violet and crystal violet as gram-positive stains; Gram's solution and compound solution of iodine U. S. P. (Lugol's solution) as differentiators; rosaniline hydrochloride, basic fuchsin, dilute carbolfuchsin and safranine as gram-negative stains, and acetone, alcohol, ether and aniline oil–xylene as decolorizers.

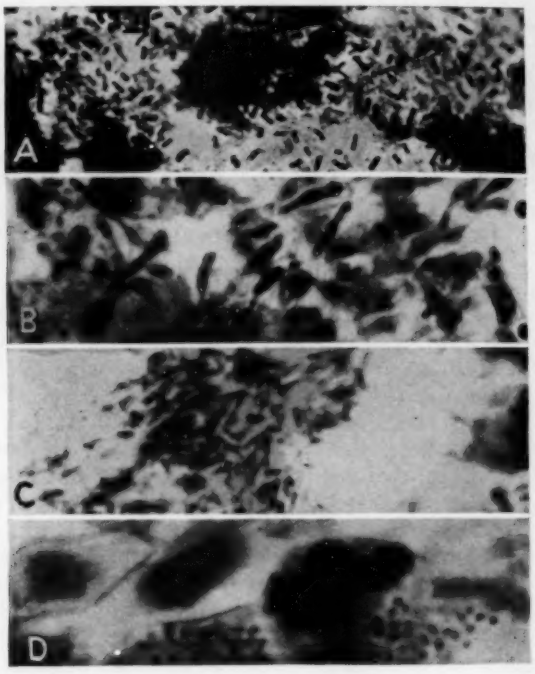
The need for rapid diagnosis led me to investigate the usefulness of a different dye and different chemicals. A new method was devised which is easily applied to frozen sections and which gives satisfactory

From Department of Pathology, Los Angeles County Hospital.

1. Gram, C.: Fortschr. d. Med. **2**:185, 1884.
2. Weigert, C.: Ztschr. f. wissensch. Mikr. **4**:512, 1887.
3. MacCallum, W. G.: J. A. M. A. **72**:193, 1919.
4. Lyon, M. W.: J. A. M. A. **75**:1017, 1920.
5. Lillie, R. D.: Arch. Path. **5**:828, 1928.
6. Brown, J. H., and Brenn, L.: Bull. Johns Hopkins Hosp. **48**:69, 1931.
7. Rudnikoff, I., and Stawsky, H.: Arch. Path. **19**:543, 1935.
8. Glynn, J. H.: Arch. Path. **20**:896, 1935.

simultaneous differential staining of gram-positive and gram-negative organisms. For example, four different frozen sections mounted on the same slide, one each of a kidney containing colon bacilli, a lung with Friedländer's bacilli, a kidney with staphylococci and a brain containing torulas, were stained, with successful differentiation of the organisms by the method described. The technic has also been used on tissues containing streptococci, Welch's bacilli, influenza bacilli, monilias, actinomycetes, meningococci, gonococci and Negri bodies.

In this new method the solutions used are: alum-hematoxylin as a nuclear stain; copper–zinc sulfate as a mordant; brilliant green as a



Differential staining of organisms in frozen sections: *A*, Friedländer's bacilli in pneumonia, stained red; × 810. *B*, monilias in kidney, stained bluish green; × 1,100. *C*, colon bacilli in pyelonephritis, stained red; × 1,620. *D*, staphylococci in pyelonephritis, stained bluish green; × 1,620.

gram-positive stain; carbolfuchsin as a gram-negative stain; dioxane (diethylene dioxide) as a differentiator, ammonium nitrate as a fortifier and creosote-xylene as a decolorizer.

THE METHOD

1. Prepare frozen sections at 7 to 10 microns in the usual manner.
2. Stain for two minutes in alum-hematoxylin (Harris' method).
3. Wash in tap water until blue and destain rapidly in acid alcohol, dipping in and out five to seven times.

4. Rinse in tap water and apply copper sulfate–zinc sulfate solution for three minutes (copper sulfate, 7 Gm. and zinc sulfate, 4 Gm., dissolved in 100 cc. of distilled water by the aid of heat).
5. Pour off; apply brilliant green solution for five minutes (0.3 Gm. of brilliant green dissolved in 10 cc. of copper–zinc sulfate mixture). Rinse in water and fortify for one minute with a 5 per cent aqueous solution of ammonium nitrate.
6. Rinse in tap water and apply carbolfuchsin (Ziehl-Neelsen method) for two minutes.
7. Rinse in tap water, blot and apply dioxane for two minutes.
8. Pour off and without washing apply creosote-xylene (equal parts), changing the solution several times and agitating the slide for even differentiation until the background appears to be clear red with no more stain leaving the section. (This step requires about one minute, and it is advisable to control the differentiation under the microscope.)
9. Clear in pure xylene two minutes.
10. Mount in gum dammar.

With the use of this method, nuclei are bluish red, gram-positive organisms bluish green, gram-negative organisms red, monilias and actinomycetes green and Negri bodies bright red with greenish chromatin bodies.

All the staining solutions are stable except the brilliant green, which keeps well about twenty-four hours.

SUMMARY

A new method for the demonstration of gram-positive and gram-negative organisms in frozen sections is presented. The staining solutions used are: alum-hematoxylin, brilliant green, copper sulfate–zinc sulfate, carbolfuchsin, dioxane, ammonium nitrate and creosote-xylene.

Forensic Medicine

THE PERCHLORATE METHOD FOR DETERMINING CONCENTRATION OF ALCOHOL IN EXPIRED AIR AS A MEDICOLEGAL TEST

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The desirability in medicolegal cases of having objective chemical evidence to supplement impressions gained from the odor of the breath or from the behavior of a person suspected of being intoxicated is well known. Although the most suitable type of specimen for such a chemical determination is the blood, there are two circumstances which may prevent blood being obtained. One is that in the absence of specific statutory authorization the withdrawal of blood may be illegal, and the other is that in order to obtain such a specimen the immediate assistance of a physician is required.

Under certain conditions and with full cooperation of the subject the analysis of a sample of urine may provide information from which the concentration of alcohol in the blood may be estimated. To obtain such a specimen it is essential that the bladder first be emptied and its contents discarded. The specimen to be tested should then be collected after the lapse of a suitable period. The concentration of alcohol in it will bear a definite relationship to the mean concentration of alcohol in the blood during the period over which that particular sample of urine was being secreted. The principal objection to the analysis of urine from the bladder as a means of determining intoxication is that it is frequently difficult to get sufficient cooperation on the part of the subject of the investigation to obtain a suitable specimen.

A third source of material is the breath. A specimen of expired air may be obtained with a minimum degree of cooperation on the part of the subject, and the results obtained from its analysis provide reliable information as to the alcoholic concentration of the blood at the time that the specimen was collected.

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In a previous communication we [1] described a new method for the measurement of alcohol in the breath which is particularly suitable to the needs of law enforcement agencies. The amount of alcohol and the amount of carbon dioxide per unit volume of expired air are determined, and the alcohol–carbon dioxide ratio thus obtained is translated into terms of blood alcohol by means of a conversion formula. The apparatus is of simple and compact construction, is inexpensive and can be readily carried in the pocket of a traffic officer. Satisfactory operation follows the most elementary instruction and requires no special skill or technical training.

It is the purpose of this investigation to examine the potential sources of error which might affect the validity of the results of such analyses and to anticipate so far as possible the various objections that might be raised in a court of law to the presentation of such results as evidence for or against intoxication.

THE BASIS OF THE FORMULA FOR TRANSLATING THE BREATH ALCOHOL–CARBON DIOXIDE RATIO INTO PERCENTAGE OF ALCOHOL IN THE BLOOD

The fact that the alcohol–carbon dioxide ratio of the breath may be used to determine the concentration of alcohol in the blood was first demonstrated by Harger [2] and is based on the following premises: According to Henry's law, the concentration of alcohol vapor in alveolar air is proportional to the concentration of alcohol in pulmonary blood. This relationship was first confirmed by Liljestrand and Linde [3] and later corroborated by Haggard and Greenberg.[4] That the carbon dioxide content of alveolar air is constant in the normal adult was first claimed by Haldane and Priestly.[5] It approximates 5.5 per cent by volume, the equivalent of about 100 mg. per liter, and has a partial pressure equal to the carbon dioxide tension in pulmonary blood. These relationships enable one to calculate the amount of alveolar air in a given sample of expired air from the measurement of the carbon dioxide in the sample, and have been used by Harger in his breath alcohol apparatus.[6] He stated

> Since the tendency of carbon dioxide to escape from the blood and other body fluids is constant, it would seem that the quantity of alcohol which accompanies the

1. Jetter, W. W.; Moore, M., and Forrester, G. C.: Am. J. Clin. Path. **11**: 75, 1941.
2. Harger, R. N.: Science **73**:10, 1931.
3. Liljestrand, G., and Linde, P.: Skandinav. Arch. f. Physiol. **60**:273, 1930.
4. Haggard, H. W., and Greenberg, L. A.: J. Pharmacol. & Exper. Therap. **52**:150, 1934.
5. Haldane, J. S., and Priestly, J. G.: J. Physiol. **32**:225, 1905.
6. Harger, R. N.; Lamb, E. B., and Hulpieu, H. R.: J. A. M. A. **110**:779, 1938.

carbon dioxide should vary directly with the concentration of alcohol in the blood . . . some of the carbon dioxide in breath represents diffusion from bronchioles and bronchi, but alcohol probably diffuses also from these surfaces in much the same ratio . . .

THE FORMULA

We have found from examination in a number of cases that the following formula is most suitable for translating the breath alcohol–carbon dioxide ratio to percentage of alcohol in the blood[7]:

$$0.2 \times \frac{\text{mg. of alcohol}}{\text{mg. of carbon dioxide}} = \text{percentage of alcohol in the blood.}$$

All breath alcohol–carbon dioxide ratios obtained in the studies presented in this paper were converted to blood alcohol percentages by means of this formula.

POTENTIAL SOURCES OF ERROR

Factors which might qualify the validity of the results may be divided into two classes: those inherent in the apparatus itself[8] and those

7. Liljestrand and Linde[3] found that 2,000 cc. of alveolar air contains the same quantity of alcohol as 1 cc. of blood. Assuming that the average concentration of carbon dioxide in alveolar air is 5.5 per cent by volume, we find that the weight of the carbon dioxide is approximately 215 mg. Thus their formula for converting the breath alcohol–carbon dioxide ratio to percentage of alcohol in the blood would be:

$$0.215 \times \frac{\text{mg. of alcohol}}{\text{mg. of carbon dioxide}} = \text{percentage of alcohol in the blood}$$

which closely duplicates the formula we arrived at experimentally. It should be noted that Harger and co-workers[6] use the weight of alcohol in 190 mg. of carbon dioxide in their conversion formula.

8. A diagram of the so-called perchlorate apparatus for the determination of breath alcohol (obtained from the Intoximeter Company, of Niagara Falls, N. Y.) is shown in figure 1. In practice the subject blows into the mouthpiece, *M*, moderately distending the balloon, *B*, which has a capacity of at least 3 liters. Backward diffusion of the breath is prevented by a check valve, *A*. The balloon assembly has another opening at the other end connected with tube *D*. The balloon assembly is so contrived that the breath sample passes immediately into the apparatus and is not held unduly in the balloon reservoir. This reduces to negligible significance the possibilities of impairment of the quality of the sample by loss of carbon dioxide through the rubber walls or absorption of alcohol vapors by condensed moisture from the breath. Tube *D* contains 5 Gm. of magnesium perchlorate ($Mg(ClO_4)_2.1H_2O$) and is connected by a U-shaped capillary tube, *F*, to tube *E* which contains 15 Gm. of ascarite (sodium hydroxide fused on asbestos). Tube *F* is of such bore that pressure from a distended balloon will force the air stream through the absorption train at the rate of 1 liter per minute. The train is kept open for about two minutes, permitting about 2 liters of breath to pass. From this sample the perchlorate first absorbs all the moisture and alcohol vapors, and then the ascarite absorbs the carbon dioxide. After the sample is taken, the apparatus is stoppered and sent to the laboratory.

physiologic and pathologic conditions sometimes encountered in persons examined which might introduce interfering factors or invalidate the premises on which the conversion formula is based.

Factors Relating to the Apparatus.—1. The condensation of moisture from the breath in the balloon assembly of the apparatus with the attendant absorption of alcohol and carbon dioxide vapors before they reach the absorption tubes.

Such an effect should be greatest at low temperatures, when the amount of condensate is largest and the solubility of gases in water greatest. To determine the possibility and significance of such errors, tests were made as follows: A number of kits were placed in the freezing compartment of a refrigerator for several hours, while others were kept at the temperature of a warm room. Tests were then made

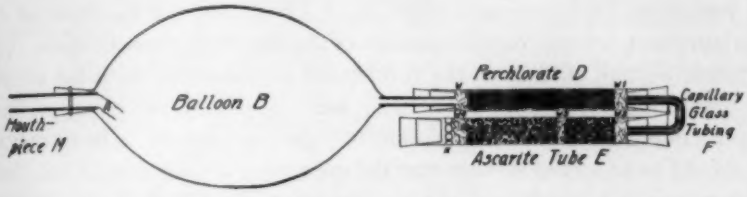

Fig. 1.—Diagram of the perchlorate apparatus for the determination of breath alcohol. W and W_1 are glass wool plugs holding the perchlorate crystals in tube D in place. The ascarite in tube E is held in place by similar plugs, W_2, W_3 and W_4. Between W_3 and W_3 is a short section containing 5 Gm. of ascarite. Between W_3 and W_4, a longer section, is 10 Gm. of ascarite, buttressed at the end by a thin perforated section of cork, K.

This arrangement affords an exact and reliable method for the absorption and subsequent determination of alcohol and carbon dioxide vapors from an air stream such as expired breath. Evidence has been previously presented showing that under the conditions of the test magnesium perchlorate completely absorbs the alcohol and moisture from a breath sample of a person with acute alcoholism and that this absorbed alcohol can be quantitatively recovered from the magnesium perchlorate by aqueous solution and distillation, with a limit of error not exceeding 2 per cent. It has also been shown that ascarite quantitatively absorbs carbon dioxide under the conditions prevailing in the test and that its entire gain in weight is due to absorption of carbon dioxide, as the passage of the air stream through the magnesium perchlorate has removed all traces of moisture.

The practicability of the apparatus is of no less importance. In its present form for field use it weighs but 4 ounces (113.5 Gm.) and is housed in a cylindric kit 8 inches (20 cm.) long and 2 inches (5 cm.) in diameter, so that it can be easily and safely carried in the pocket. Proper use of the apparatus involves a minimum of manipulation, and samples can be taken by a person with very little preliminary instruction. The sample can be analyzed immediately in the laboratory in about forty minutes, or, if the situation requires, it can be preserved indefinitely without deterioration.

on persons suffering from acute alcoholism in the warm room with the kits of corresponding temperature, and other tests were conducted on the same persons within a few minutes at 15 F. with the refrigerated kits. There was no visible condensate in the apparatus at the warm temperatures but considerable quantities of condensed moisture collected as droplets in the balloon assembly when the tests were made in the cold. However, no significant differences in the breath alcohol determinations of the two series were found, and it is felt that no error due to such condensation at low temperatures is incurred.

By way of understanding these results, one considers the fact that the partial pressure of alcohol vapors in breath is always so small that direct condensation of alcohol cannot be effected by the most extreme winter temperatures. Carbon dioxide is a permanent gas under all conditions encountered. Accordingly, removal of either of these components of breath in the condensate depends on, first, the condensation of the moisture and, second, on the solution of the vapors in these droplets. The extremely small surface of the condensate as compared with the volume of breath employed and the relative lack of affinity for either of these vapors make the loss by absorption of either so slow as to be negligible. It should be observed further that the apparatus is contrived to keep such absorption at a minimum. In its operation the breath begins to pass into the absorption tubes as soon as blown into the balloon. The flow is completed at the end of two minutes. A total of 2 liters or more of breath is blown into the balloon; so the average exposure is only one minute or less, a period which appears to be too brief for measurable effects to accumulate.

2. Loss of alcohol or carbon dioxide vapors by leakage or absorption in the balloon.

It has been stated by Haggard and Greenberg [9] that rubber absorbs alcohol and that half the alcohol in such a balloon would be absorbed in a few minutes. Further, it is well known that carbon dioxide dissolves in rubber and readily passes through thin rubber membranes. Both phenomena have been demonstrated experimentally. However, as stated in the immediately preceding paragraph, the technic of passing the breath immediately through the balloon to the absorption tubes obviates effectively a detectable loss of either constituent. Tests in which the expired breath was caused to be held for varying periods in the balloon before being allowed to pass through the absorption tubes required minutes for a positive effect to be obtained, and when this was observable the experiment always yielded a result lower than that obtained by analysis of the blood. When the period of storage was

9. Haggard, H. W.; Greenberg, L. A., and Cohen, L. H.: New England J. Med. **279**:466, 1938.

as much as five minutes in addition to the normal time required for passage, the apparent loss of alcohol amounted to 15 per cent of the total. With ten minutes' storage the loss approximated 30 per cent. Simultaneous losses of alcohol and carbon dioxide would be compensating errors, but it appears from the experiments that the loss of the alcohol would be the more significant. However, the many routine tests conducted in the manner prescribed for the operation of the apparatus prove that no detectable error in determining the ratio of the two substances in the air as expired need be introduced.

An elementary criterion of the value of any analytic method is the performance of that method and particularly the duplicability of the results when different technicians apply the method to the same subject

TABLE 1.—*The Results of Duplicate Breath Tests (the Results Are Given as Converted to Percentages of Alcohol in Blood)*

.027	.144	.150
.028	.145	.155
.032	.151	.158
.029	.154	.162
.045	.133	
.048	.134	
.049	.132	
.052	.133	
.055	.125	
.058	.133	
.060	.131	
.062	.135	
.113		
.112		
.127		
.134		
.132		
.139		

matter under different conditions. Tests in duplicate on several persons with acute alcoholism show that the apparatus yields good checks (table 1).

Factors Relating to the Test Subject.—Apart from the physical and chemical limitations of the apparatus and of the method itself there is the possibility that physiologic differences in test subjects might introduce errors in the determination of the amount of alcohol present or render the conversion formula inapplicable by invalidating the premises on which it is based. For instance, there may be present in the breath reducing materials other than ethyl alcohol which would be absorbed by the perchlorate and be reported in the subsequent analysis as alcohol. Also the alveolar carbon dioxide tension of the subject may depart from the normal.

1. Volatile reducing substances in the breath other than ethyl alcohol.

In a fair number of tests on persons who did not have acute alcoholism, made under a variety of conditions, including hyperventilation, acute fatigue and emesis, no reducing matter was found in the perchlorate absorbent. From persons in severe diabetic acidosis but not suffering from acute alcoholism a reducing body (acetone) was absorbed by the perchlorate, but the amount as measured by this analytic procedure did not exceed the equivalent of 0.01 per cent alcohol. The presence of acetone, however, does indicate a profound disturbance in the acid-base equilibrium and a consequent abnormality in the carbon dioxide tension of the blood which would affect the meaning of the alcohol–carbon dioxide ratio. This condition will be discussed in a succeeding paragraph.

(*a*) Paraldehyde. The following experiment was performed to determine the reducing effects of paraldehyde in the expired breath.

A man aged 50 weighing 160 pounds (72.5 Kg.), who did not have acute alcoholism, was given 16 cc. of paraldehyde at 8:30 a. m. A test for breath alcohol made before the administration of paraldehyde gave a practically negative result (the equivalent of 0.0018 per cent blood alcohol). At 10 a. m. the patient was sleeping soundly and was awakened with slight difficulty. The odor of paraldehyde on the breath was distinct, but the quantity of reducing matter in the breath had increased to the equivalent of only 0.0033 per cent blood alcohol. At 11:45 a. m. the patient was sleeping soundly and was awakened for a breath test and given 16 cc. of more of paraldehyde. The breath test showed the equivalent of 0.0093 per cent blood alcohol. At 2 p. m. the patient was sleeping soundly with stertorous breathing and was awakened with difficulty for a breath test. This showed the equivalent of 0.0119 per cent blood alcohol. At 4 p. m. the sleep was less profound, and the result of the breath test at this time was only 0.0134 per cent.

It may be concluded, therefore, that although paraldehyde is capable of imparting limited reducing properties to the breath, its physiologic effect as a soporific is not accompanied by the elaboration of a sufficient quantity of reducing agent in the expired air to affect significantly a test for breath alcohol.

(*b*) Esters, aldehydes and other substances in alcoholic beverages. The characteristic nuances in body, flavor and bouquet of alcoholic beverages are attributed to esters, aldehydes, acids and other constituents developed in the fermenting, distilling and aging arts. Quantitatively, however, these components rarely exceed 0.5 per cent of the ethyl alcohol content of the beverage, and their contribution to the reducing properties of the breath is comparatively insignificant, i. e., 0.5 per cent of the total.[10]

(*c*) Methyl alcohol. In spite of the poisonous nature of this homologue in the alcohol series, it is sometimes an ingredient in illegally prepared beverages. When absorbed from the breath and analytically

10. Thorpe, E.: Dictionary of Applied Chemistry, New York, Longmans, Green & Company, 1912.

determined by this apparatus, 1 unit of methyl alcohol is the equivalent of 1.44 units of ethyl alcohol by weight.[11]

2. Variations from normal alveolar carbon dioxide tension.

The ultimate reliability of the application of the breath alcohol–carbon dioxide ratio to the determination of the alcoholic content of the blood depends on the determination of a variable (alcohol) in terms of a presumed constant (carbon dioxide). An examination of published physiologic data concerning alveolar carbon dioxide tension shows (*a*) that this tension is constant for any one person under normal conditions, (*b*) that it differs from one person to another and (*c*) that it may fluctuate widely in any person under abnormal conditions.

These conclusions have been corroborated indirectly by determining the breath alcohol–carbon dioxide ratios of persons with acute alcoholism when experimental conditions were caused to vary within such short periods that the alcohol content of the blood could be regarded as constant, so that any fluctuation in the ratio reflected the changing value of the carbon dioxide member as a result of changing conditions:

(*a*) The tension of alveolar carbon dioxide for any one person is constant under normal conditions: table 1 shows the close agreement of determinations when two breath samples were taken from a person with acute alcoholism within periods not exceeding four minutes. For practical purposes the blood alcohol level in each case must be regarded as constant, and these findings indicate that the carbon dioxide pressure must also have been constant during the periods of the tests. These observations are consistent with the findings of Haldane and Priestly [5] and of Fitzgerald and Haldane.[12] The former have stated ". . . for each individual the normal alveolar CO_2 pressure appears to be an extraordinarily sharply defined physiological constant."

On the other hand, Dodds [13] has noted an increase in the carbon dioxide tension following the ingestion of food. The greatest percentage increase was approximately 14; the average was in the neighborhood of 5. The effect was transitory, lasting some two to three hours, and was not cumulative, as the ingestion of more food within that time produced no further increase. Our data have not been accumulated

11. The qualitative test used for the detection of methyl alcohol in the perchlorate distillate is as follows: Methyl alcohol is oxidized to formaldehyde by plunging a heated copper wire into the solution several times. When formaldehyde is present a red color is produced on the addition of phloroglucinol reagent. The reagent is prepared by dissolving 1.0 Gm. of phloroglucinol in 100 cc. of a 10 per cent solution of sodium hydroxide; 0.1 cc. of this reagent is used for each 1 cc. of solution.

12. Fitzgerald, M. P., and Haldane, J. S.: J. Physiol. **32**:486, 1905.

13. Dodds, cited by Wright, S.: Applied Physiology, New York, Oxford University Press, 1936.

with this effect in mind. However, we have followed several persons who ingested alcohol over a period of eight hours, during which time at least one meal was taken, and were unable to detect an increase in the carbon dioxide tension. It is acknowledged, however, that this procedure for determining the alveolar carbon dioxide pressure is indirect and yields but an approximation as compared with direct measurement on alveolar air when truly representative samples are obtained.

(*b*) Variation in alveolar carbon dioxide pressure from person to person: For the alcohol–carbon dioxide ratio to be a generally accurate measure of the quantity of alcohol in a given volume of alveolar air, and hence indirectly of the blood alcohol, the pressure of carbon dioxide in the alveolar air must be the same in all persons. Fitzgerald and Haldane [12] found this value in a group of normal adult men to range from 4.7 to 6.3 per cent by volume. While their average (5.5 per cent) approximates the value used in our formula for translating the breath alcohol–carbon dioxide ratio to percentage of blood alcohol, it is evident that a significant variation from this average may occur, and this indicates that it is not possible to predict the alveolar carbon dioxide pressure of a given man within narrower limits than $\pm$ 15 per cent ($\frac{47}{55}$ = 85.5 per cent, $\frac{63}{55}$ = 114.5 per cent). The majority of cases was found to fall well within these extremes, but it must be conceded that a variation up to $\pm$ 15 per cent from the expected alveolar carbon dioxide tension may occur.

In view of this it would be expected that a variation of like magnitude would be found on comparing the breath alcohol–carbon dioxide ratios in a large number of cases with blood alcohol determinations made simultaneously. Such a variation has been found. As shown in figure 2, which graphically summarizes data discussed in greater detail later, it is seen that the extreme variations between the percentage of blood alcohol as calculated from the breath alcohol–carbon dioxide ratio and the percentage of blood alcohol as determined by chemical analysis approximates 16 per cent. The average of all correlations is in the neighborhood of 6.6 per cent.

(*c*) Fluctuation of the alveolar carbon dioxide tension of the individual under abnormal conditions: In order to establish the validity of the breath alcohol–carbon dioxide ratio for determining the concentration of alcohol in the blood it is important to recognize those conditions that are capable of producing a variation in the alveolar carbon dioxide pressure. Among these are acute alcoholism, hyperventilation, muscular exercise and variations in acid-base equilibrium.

As to acute alcoholism, it appears that the effect of alcohol on alveolar carbon dioxide tension is negligible until the stage of narcosis

is reached. Then the respiratory center is depressed with a consequent elevation of the alveolar carbon dioxide. The latter effect has not been observed in our series of tests, as none of the determinations has fallen in the extremely high brackets of blood alcohol concentration. From the standpoint of the purpose of this method and this apparatus, interest in such a condition is largely academic. A person in stupor cannot operate an automobile or blow into the balloon; in any event, an effect encountered of lesser degree favors the accused, as the breath alcohol results would be in error on the low side.

Reduction of the alveolar carbon dioxide pressure by pulmonary hyperventilation is well known. We have found that this condition

TABLE 2.—*Effect of Hyperventilation on the Breath Alcohol–Carbon Dioxide Ratio*

Subject	1	2	3	4
Control blood alcohol	.127	.051	.128	.112
Control breath alcohol	.129	.047	....	.100
Breath alcohol immediately after hyperventilation	.251	.083	.215	.151

TABLE 3.—*Effect of Hyperventilation on the Breath Alcohol–Carbon Dioxide Ratio*

Control sample immediately before hyperventilation	.133
Hyperventilation of about 2-3 minutes	
Sample immediately after hyperventilation	.254
Sample 2½ minutes after hyperventilation	.136
Sample 5 minutes after hyperventilation	.134
Sample 7½ minutes after hyperventilation	.123
Sample 12 minutes after hyperventilation	.132
Sample 21 minutes after hyperventilation	.131
Sample 24 minutes after hyperventilation	.141
Sample 34 minutes after hyperventilation	.135
Sample 39 minutes after hyperventilation	.125
Sample 44 minutes after hyperventilation	.122
Sample 49 minutes after hyperventilation	.127

appreciably affects the alcohol–carbon dioxide ratio. Alcohol was given to 4 volunteers. After the collection of control blood samples and the making of tests for breath alcohol, the subjects voluntarily hyperventilated themselves to the point of imminent syncope. Thereupon breath samples were again taken. The results (table 2) showed an increase of from 50 to 100 per cent over the control samples, which must be interpreted as due to a corresponding decrease in the alveolar carbon dioxide tension.

In order to determine how long the effect of hyperventilation persists the observations were continued for a period of fifty minutes on other subjects. Table 3 shows a typical example. It is observed that two and one-half minutes after hyperventilation the breath alcohol had returned to the level of the control. This was coincident with the return to normal breathing after the period of apnea immediately following

hyperventilation. Other tests have shown similar results, and in all cases equilibrium has been attained within five minutes after hyperventilation.

When the apparatus is used, the subject usually takes a deep breath before blowing into the balloon. Experiments have been conducted in which the subjects have taken two, four and six deep inhalations before blowing into the balloon. In no instance was any variation detected following two or four deep inspirations. Mild transitory effects were found after six deep breaths.

A device was used wherewith the first 500 to 700 cc. of the expired breath was wasted and a more truly representative sample of alveolar air was obtained. The results agreed with those obtained in the normal manner. It is to be expected that the quantities of alcohol vapors and carbon dioxide in the dead air would be less than in alveolar air, but the ratio remains the same.

To determine the effect of muscular exercise, an experiment was made as follows: With each of 6 male subjects who had ingested alcohol, control samples were taken for determining alcohol content by both blood analysis and the perchlorate method. Then the subject performed violent muscular exercises for from three to five minutes to the point of acute fatigue. The blood alcohol content as translated from the breath alcohol–carbon dioxide ratio determined immediately after exercise appeared to decrease about 20 per cent in 3 of the cases. In the other 3 cases the lowering was negligible. This appears to indicate that the alveolar carbon dioxide tension had been increased by the violent exercise and is in accord with the statement of Haldane and Priestly [5] ". . . during muscular exercise the alveolar CO_2 tension is raised slightly with a correspondingly large increase in the lung ventilation." Breathing becomes normal again in a few minutes, signaling the return of alveolar carbon dioxide tension to normal. No effect was observed when the subjects performed light exercise, as walking moderately on level ground for periods up to an hour, but any effect produced would favor the subject in yielding a result in error on the low side.

One of the compensatory adjustments which is brought into effect by abnormal hydrogen ion concentration in the blood is a change in the alveolar carbon dioxide tension. When the hydrogen ion concentration increases, the alveolar carbon dioxide is also increased, and vice versa.

There is a tendency toward alkalemia during gastric hydrochloric acid secretion. A diet high in proteins produces the same effect. The secretion of alkaline digestive juices or the ingestion of a vegetable diet has the opposite effect.

These changes in alveolar carbon dioxide pressure, however, are apparently of small magnitude, since we have been able to detect no definite influence on the alcohol–carbon dioxide ratio of subjects whom

we have followed for periods up to eight hours, during which time two meals had been taken.

The effects of acid and basic salts, such as ammonium chloride and sodium bicarbonate, have been only partially explored. Ingestion of large quantities of such substances can produce definite acidosis or alkalosis. However, no variations have been observed after the ingestion of 5 grains (0.32 Gm.) of ammonium chloride thrice daily for three days previous to experimental tests.

The severe acidosis occurring in starvation, uncontrolled diabetes and uremia is associated with a marked decrease in the alveolar carbon dioxide tension. Several persons addicted to alcohol who were admitted to the Washingtonian Hospital, Boston, in severe starvation acidosis were examined by blood and breath analysis. The blood alcohol content as calculated from the breath alcohol–carbon dioxide ratio was considerably

TABLE 4.—*Effect of Emesis on the Breath Alcohol–Carbon Dioxide Ratio*

	Breath	Blood
Control samples before emesis	.143	.138
	.142	
Emesis		
Immediately after emesis	.148	
5 minutes after emesis	.126	
10 minutes after emesis	0.95	
15 minutes after emesis	.131	
20 minutes after emesis	.142	
30 minutes after emesis	.148	.132

higher than the actual concentration as determined by blood analysis. In this respect the results were similar to those encountered in hyperventilation. Practically all types of acidosis can be detected (with the possible exception of uremia) by the presence of acetone in the breath. Since actone is absorbed in perchlorate as rapidly and perfectly as alcohol has been shown to be, it is a routine procedure to test all subjects qualitatively for this substance [14] and reject the breath alcohol–carbon

14. The residue of the distillate from the perchlorate after the 5 cc. samples have been titrated for alcohol amounts to 30 cc. or more and contains 60 per cent or better of the alcohol, acetone and other vapors which were absorbed from the breath. It is put in a 50 cc. flask under a 14 inch (35.5 cm.) Young fractionating column and slowly brought to boil. The heat is regulated so that the condensation ring ascends the column slowly. The first cubic centimeter is caught in a small test tube and 4 drops of a 10 per cent solution of salicylaldehyde in ethyl alcohol added and well mixed. Then a small pellet of potassium hydroxide is added and the solution warmed without further disturbance. The presence of acetone is denoted by the appearance of a red ring above the potassium hydroxide layer. If a parallel blank is run, the presence of 0.1 mg. of acetone in 1 Gm. of blood can be readily detected.

dioxide ratio as a quantitative measure of blood alcohol if acetone is found to be present.

An elevation of alveolar carbon dioxide tension occurs in alkalosis. Of practical significance for the purposes of this apparatus is the alkalosis that may result from emesis. For experimental purposes, control blood and breath samples were taken from a subject, and emesis was then induced and was carried to the point at which complete evacuation of the stomach was thought to have been attained (table 4). It will be observed that the effect was not immediate, but at five and ten minutes after emesis the apparent blood alcohol percentage as calculated from the

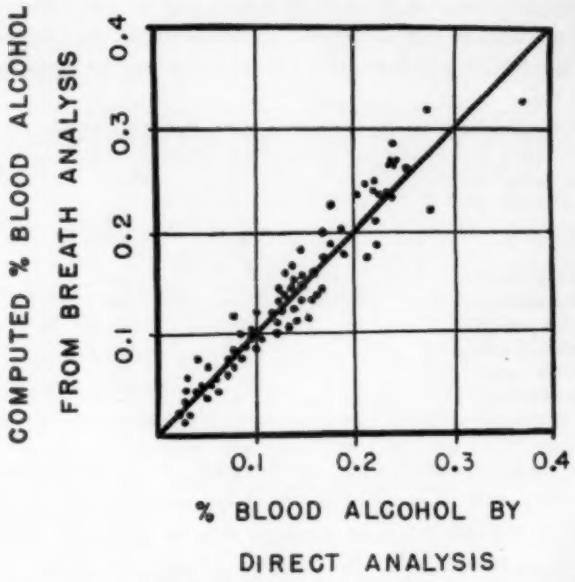

Fig. 2.—Correlation of the results of breath and blood analyses in 79 cases.

breath alcohol–carbon dioxide ratio had been depressed 15 and 35 per cent, respectively. After fifteen minutes the effect had passed and there was again good agreement between the two methods, which continued to the end of the experiment. It is noted that the error is again in favor of the subject examined.

In other experiments we have found that the effect may persist as long as twenty-five minutes. Confusing results have been encountered which are traceable to the hyperventilation which may accompany vomiting. For most accurate results, a waiting period of thirty minutes is required if emesis has occurred, but any error introduced favors the subject in that the calculated alcoholic content is on the low side.

CORRELATION OF THE RESULTS OBTAINED BY BREATH AND BLOOD ANALYSES IN A SERIES OF ROUTINE CASES

Blood alcohol content was determined both by the breath alcohol–carbon dioxide ratio and by analysis of the blood in 32 cases. The subjects were men ranging in age from 20 to 60 years. About half of them were volunteers, and the tests were taken in conjunction with the accumulation of other experimental data. The remaining subjects were patients admitted to the Washingtonian Hospital, Boston. A total of 79 breath-blood pairs of samples were obtained from the 32 men. No more than 3 tests were made on a single subject, and in all cases an interval of at least one hour elapsed between successive tests.

All breath tests were made according to the procedure previously described. Figure 2 includes all the results of these tests except those in which acetone was detected in the perchlorate distillate. For this figure the alcohol–carbon dioxide ratio in each case has been converted to percentage of blood alcohol by the conversion formula previously given, in order that a comparison might be made with the concentration of alcohol in the blood as determined by direct analysis.

It will be seen that the correlation between the results of blood and breath analysis was good. The maximum deviation observed between the results of breath and blood analysis was 16 per cent. Most of the high percentile deviations occurred when the absolute amount of alcohol was small. It is felt that the examination of a large number of routine traffic cases in a manner similar to that in the 79 reported here would be a conclusive measure of the worth of this method.

COMMENT

In this paper a critical evaluation of the perchlorate method for the determination of breath alcohol has been presented. It has been shown that loss of alcohol or of carbon dioxide before the breath reaches the absorption tubes is not significant when the apparatus is used in accordance with instructions. Reducing substances which may be present in the breath and which would be measured as alcohol by this method have been examined and found to be unimportant within the intended uses of this apparatus. Among those considered are the reducing substances normally present in the breath, also acetone, paraldehyde, the esters, aldehydes and other constituents of beverages, and methyl alcohol.

The conditions which may cause the tension of carbon dioxide in alveolar air to depart from normal and invalidate in a corresponding measure the formula for converting the breath alcohol–carbon dioxide ratio to percentage of blood alcohol have been examined. It is concluded that under normal conditions the carbon dioxide tension of a normal adult man may vary as much as 15 per cent from the average

used in our conversion formula. On this score a departure of 15 per cent from the true percentage of the alcohol in the blood may be encountered in a determination by means of the breath alcohol–carbon dioxide ratio. The maximum deviation encountered in our series of 79 cases did not exceed 16 per cent. However, the absolute deviations for all reasons from the results of blood analyses averaged 6.65 per cent, and the algebraic average of all results was 0.8 per cent above the results of blood analyses; i. e., the high and low errors balanced each other almost exactly.

Various abnormal conditions may disturb the accuracy of the method, but within the purposes for which the method is devised, they can be readily avoided. The pharmacologic effect of alcohol on the respiratory center itself is not important within the range of usefulness of the method. The effect of hyperventilation is marked but very transitory and is avoided by making the test after the subject has quieted. Normal exercise does not influence the result, and the effect of violent exercise is dissipated with the return of normal breathing, when the test should be made.

Acidosis and alkalemia temporarily induced by alimentary processes appear to be unimportant in the operation of these tests. An exception is the alkalosis which follows emesis. This disturbance may persist for some time, and a waiting period of thirty minutes after emesis is recommended. Severe acidosis accompanied by persistent hyperventilation is revealed by the routine test for acetone in the perchlorate tube. In such a case the conversion formula will indicate a level of blood alcohol higher than actually exists. In extreme cases this may be 100 per cent above the true level, and the method becomes a qualitative test for alcohol, quantitative only within very elastic limits.

SUMMARY

A critical evaluation of the perchlorate method for the determination of breath alcohol has been made, and the following data have been presented: When the test is properly made there is no loss of alcohol or of carbon dioxide before the breath reaches the absorption tubes; reducing substances in the breath other than alcohol are negligible in their effect; the presence of acidosis or alkalosis in the test subject materially affects results, but these conditions may be controlled or eliminated by proper precautionary measures.

In a series of 79 cases in which breath and direct blood analyses were made, the deviation did not exceed 16 per cent in any instance.

In view of the simplicity of operation and the ease with which the instrument can be transported, this method is well adapted for use by law enforcement agencies.

General Reviews

TUMORS OF THE SPINAL CORD

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Tumor of the spinal cord is much more common than usually is realized, for approximately 1 of every 5 neoplasms of the central nervous system is a tumor of the spinal cord. Because of this fact it seems worth while to review some of the literature on the histogenesis, microscopic appearance, distribution and frequency of tumors of the spinal cord and its membranes. It is impossible to discuss the voluminous literature on the subject, since the majority of the reports deal with single cases in which either the pathologic studies were inadequate or added little to understanding of the tumors. In this review only reports of cases which were unusual or in which the studies developed a new observation or hypothesis will be considered.

Interest in tumors of the spinal cord has increased since it was found that most of them could be removed surgically. This became possible because of increased accuracy in locating the sites of the neoplasms. Roentgenograms taken after the introduction of radiopaque iodized oil into the subarachnoid space demonstrated clearly the levels of neoplasms. More recently, the injection of air has extended the use of roentgenography as a diagnostic aid, since air is considered less irritating to the meninges. Studies of the cerebrospinal fluid are also of help in differentiating between a neoplasm and an inflammatory lesion.

Tumors that arise in the spinal cord or the meninges or that are attached to the nerve roots within the spinal canal usually are referred to as "tumors of the spinal cord." Penfield [1] and Penfield and Young,[2] however, have used the term "tumors of the sheaths of the nervous system" in order to include meningeal fibroblastoma and perineural fibroblastoma in contrast to "primary tumors of the spinal cord and

From the Sections on Pathologic Anatomy and Surgical Pathology of the Mayo Clinic.

1. Penfield, W.: (*a*) Surg., Gynec. & Obst. **45**:178, 1927; (*b*) Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932, vol. 3, pp. 953-990.

2. Penfield, W., and Young, A. W.: Arch. Neurol. & Psychiat. **23**:320, 1930.

intradural filum terminale." The latter term was used by me in order to include glioma and other types of intramedullary tumors.

Tumors of the spinal cord may be classified by types of tumors or by levels of the spinal cord at which they are found, such as cervical, thoracic and so forth. However, neurosurgeons commonly subdivide them into extradural and intradural, and extramedullary and intramedullary, tumors; the last method is the one used most commonly.

Since the histologic structures of the spinal cord and its coverings are the same as those of the brain and its coverings, similar types of tumors are found in both places but with varying frequency. The spinal cord is a small structure which is injured easily. It would be expected, therefore, that the duration of the tumors which involve it, or rather the duration of symptoms of which the patients complain, would be relatively short, but, strangely enough, this is not so. The symptoms of intramedullary tumors are of longer duration than those of extramedullary tumors. This may be attributable, at least in part, to the fact that extramedullary neoplasms may grow considerably before they compress the spinal cord sufficiently to produce symptoms.

The commonest site of tumors of the spinal cord is the thoracic segment. Although this is the longest portion of the spinal cord, the number of cases in which it is involved is even higher than its greater length would indicate. An adequate explanation to account for this discrepancy has not been offered.

Extramedullary tumors occur at any site around the cord; most of them, however, are found posterior to the spinal medulla. Meningioma may be situated anterior to the spinal cord but occurs more frequently on a lateral aspect, about a nerve root but not attached to it. The explanation for this is given when the histogenesis of meningioma is discussed. Neurofibroma usually arises from, and is attached to, a posterior nerve root, although occasionally it is found attached to an anterior nerve root. This location of the more common tumors of the spinal cord renders them more readily accessible to the surgeon.

Most neoplasms of the spinal cord are malignant in the sense that they steadily and slowly increase in size. The grade of malignancy, however, is usually low. Many authorities refer to meningioma and neurofibroma as benign tumors. Compared with neoplasms found elsewhere in the body, they are relatively benign; however, they increase in size and will cause death unless they are removed completely. A small percentage of the tumors in each of these groups are actively malignant, with a short duration and marked cytologic activity. Highly malignant tumors of the spinal cord never metastasize to other organs or outside the subarachnoid space, which may be extensively invaded by some of these malignant neoplasms; some forms of meningioma extend through

the dura mater. From the surgical point of view, most of these neoplasms are benign.

Tumors of the spinal cord occur most commonly in persons in the middle years of life and rarely among the young or the aged. The evidence available indicates that trauma plays little role in their etiology. This statement holds true for neoplasms but not for some specially selected lesions which could be included with the tumors. During recent years there has been much interest in the condition frequently referred to as protruded vertebral disk, ruptured disk and herniation of the nucleus pulposus. In many ways this lesion simulates a tumor of the spinal cord, especially if the protrusion is large. Recent evidence indicates that trauma or strain plays a major role in the etiology of protrusion of a vertebral disk in a high percentage of cases. However, it is not yet clear whether there is not some predisposing cause, such as a congenital or an acquired degeneration or weakness of the annulus fibrosus that allows protrusion to occur.

It is an accepted fact that there is a close relationship between trauma and certain forms of meningioma of the brain, but such a relationship cannot be found between trauma and meningioma of the spinal cord.

More than 70 per cent of the tumors of the spinal cord belong to three main groups, namely, meningioma, neurofibroma and the intramedullary tumors; i. e., most of the neoplasms arise from the meninges, from the nerve roots or from the substance of the spinal cord or its prolongation downward, the filum terminale. The remaining 30 per cent comprise neoplasms of varying types, such as blood vessel tumors, sarcoma, chordoma, teratoma and so forth.

MENINGIOMA

Genesis.—Meningioma, as the name suggests, designates that group of neoplasms which arise from the meninges. Use of this term avoids the discussion as to whether the cells from which the tumors arise are mesodermal or neuroectodermal, i. e., whether the neoplasms belong to the sarcoma group or the glioma group of tumors. Earlier workers referred to these tumors as psammoma and sarcoma of the dura mater. Schmidt [3] was the first to point out that in reality these tumors arise from the arachnoid and from the pacchionian bodies. He referred to the cells of the arachnoid as endothelial cells, and thus the tumors were classified as endothelioma. He demonstrated that meningioma is found most frequently where pacchionian bodies are most numerous. Weed [4] concluded, after a study of the embryos of human beings and swine, that the subarachnoid space is produced by breaking down of the perimedul-

3. Schmidt, M. B.: Virchows Arch. f. path. Anat. **170**:420, 1902.

4. Weed, L. H.: Contrib. Embryol. **5**:1, 1917.

lary syncytium and dilatation of the existing mesenchymal spaces. He also expressed the opinion that the dura mater, arachnoid and pia mater develop from the perimedullary mesenchyme. In a later study of the cells of the arachnoid Weed [5] found clusters of cells on this membrane and concluded that they were mesothelial cells, that they were the forerunners of endothelioma and that they were indicative of age. He found 2 early tumors of this type in a series of cats and pointed out that the cells of these small tumors were morphologically indistinguishable from the arachnoid cells. He noted also that these small tumors had beginning attachments to the dura mater.

Mallory,[6] in a thorough morphologic study of surgically removed tumors diagnosed as meningioma, found in carefully prepared preparations of 1 tumor that each cell was surrounded by fibroglia fibrils and that these were more prominent than the collagen. Fibroglia fibrils and collagen are the distinguishing characteristics of the type of cell known as the fibroblast, and thus the tumor is diagnosed as fibroblastoma. He was of the opinion that the elastic tissue present came from the dura mater and large blood vessels. Fibrous whorls often become calcified and in this way produce the so-called psammoma bodies. This type of tumor invades the dura mater, the skull and even soft tissues outside the skull, but apparently it never invades the brain or the nonvascular arachnoid from which it arose. Penfield [1b] also stated that meningioma may arise from any portion of the meninges but that it is derived most frequently from arachnoid tufts growing into, and being vascularized by, the dura mater. Whorls form around collagen, small vessels or other structures; degeneration occurs in the centers of these whorls, and with the deposition of calcium psammoma bodies are formed. At times it is difficult to demonstrate fibroglia fibrils, but they can be found on persistent search. In the more slowly growing types of meningioma the neoplastic cells themselves may form collagen; this slowly growing type of meningeal fibroblastoma is typical of the tumors found in the spinal canal.

Bailey and Bucy [7] tended to agree with Mallory and Penfield that the meningeal tumors were of fibroblastic origin, although they did so with some reservations. They said:

> . . . meningeal tumors are overwhelmingly of the nature of connective tissue, which does not necessarily mean that they are mesodermal. The theory of the specificity of the three germ layers is no longer accepted by embryologists. All of the mesenchyme of the body is derived ultimately from epithelium, either from the entoderm or the ectoderm, usually apparently from the region of the junction of these two germ-layers.

5. Weed, L. H.: Bull. Johns Hopkins Hosp. **31**:343, 1920.
6. Mallory, F. B.: J. M. Research **41**:349, 1920.
7. Bailey, P., and Bucy, P. C.: Am. J. Cancer **15**:15, 1931.

Globus,[8] after a morphologic study of embryos and meningioma, stated that meningioma is mesodermal in origin and that few now believe that the meninges arise from nerve tissue or the neural crest. In their recent book on meningioma Cushing and Eisenhardt [9] stated that meningioma arises from the meningocyte, but they did not state whether this cell is epiblastic or mesoblastic; they said, however, "it begins to be reasonably certain that meningiomas and neurinomas are at least cousins with a common epiblastic paternity."

Oberling [10] agreed with Strasser [11] and elaborated on the hypothesis that the pia-arachnoid is one syncytial membrane. He distinguished two cells, one a larger cell, which he called a meningoblast, and the other a smaller cell, similar to the mesenchymal elements. He expressed the opinion that the larger cells migrated from the neural crest to the meninges. This idea was received enthusiastically by Roussy and Cornil [12] and others.

All of the aforementioned studies and conclusions were dependent on specific staining methods and the appearances of cells. Harvey and Burr [13] performed transplantation experiments on the embryos of Amblystoma punctatum at a specific stage in the development of these organisms and stated that the view of earlier workers, namely, that the pachymeninx and the leptomeninx have a common origin in a primitive mesenchyme derived from a specific germ layer, is incorrect. They stated that certain ectodermal elements, derived in large part from the neural crest, are contributed to the mesenchyme and take part in the formation of the leptomeninx. Such an origin suggests that the cells of the leptomeninges may have certain characteristics of their own, apparent in their reaction to injury and in the neoplasms arising from them.

The experiments of Harvey and Burr were repeated by Flexner,[14] who could not confirm their results. Harvey, Burr and Van Campenhout [15] carried out some further experimental work with chickens and confirmed their previous observations and conclusions. The cells on the outer surface of the arachnoid generally are considered the type cell of meningioma. The arachnoid cells collect in clusters at the tips of

8. Globus, J. H.: Arch. Neurol. & Psychiat. **38**:667, 1937.

9. Cushing, H., and Eisenhardt, L.: Meningiomas: Their Classification, Regional Behavior, Life History, and Surgical End Results, Springfield, Ill., Charles C. Thomas, Publisher, 1938.

10. Oberling, C.: Bull. Assoc. franç. p. l'étude du cancer **11**:365, 1922.

11. Strasser, cited by Oberling.[10]

12. Roussy, G., and Cornil, L.: Ann. d'anat. path. **2**:63, 1925.

13. Harvey, S. C., and Burr, H. S.: Arch. Neurol. & Psychiat. **15**:545, 1926.

14. Flexner, L. B.: Contrib. Embryol. **20**:31, 1929.

15. Harvey, S. C.; Burr, H. S., and Van Campenhout, E.: Proc. Soc. Exper. Biol. & Med. **28**:974, 1931.

arachnoid villi and are considered a common site of origin of meningioma. Elman [16] and later Hassin [17] revealed that arachnoid villi and cell coverings similar to those of the cerebral meninges occur along the spinal cord. The villi are most numerous near the sites at which the nerve roots emerge through the arachnoid and dura mater. This finding explains why meningioma of the spinal canal is in most instances found about a nerve root but not attached to it.

Foot [18] recently stated that meningioma arises from the meningocytes of the arachnoid villi. These meningocytes are the "cap" cells of the arachnoid villi and arise from the neural crest. Thus meningioma is neuroectodermal in origin. However, Foot was of the opinion that meningioma is composite in makeup, containing meningocytes and stromal cells from mesenchymal elements of the pia-arachnoid or dura mater, to which meningioma in most instances becomes attached. Usually, meningocytes predominate and probably represent the type cell of meningioma. O. T. Bailey [19] also stated that the more modern evidence on the origin of arachnoid cells (meningocytes) favors the neural crest but that a complicated stroma is present which is necessary for the nutrition of the arachnoid cells. The stroma contains several elements, such as collagen, blood vessels and fibroglia, which come from fibroblasts and not from tumor cells.

Classification.—Several attempts have been made in recent years to subdivide tumors classified as meningioma into subgroups. Bailey and Bucy were the first, and they seem to have the simplest classification. They have nine subdivisions as follows: (1) the mesenchymal type of meningioma, i. e., loose connective tissue with numerous blood vessels and abundant reticulum spreading from the blood vessels throughout the tumor; (2) the angioblastic type; (3) the meningiotheliomatous type, which is the most common variety of meningioma, presenting closely packed large cells, sometimes forming whorls, or lobules with thick, vascular stroma; (4) the psammomatous type, which is similar to the third type except that there are psammoma bodies with calcium and iron; (5) the osteoblastic type; (6) the fibroblastic type, with fusiform cells running in small bundles and with numerous mitotic figures; (7) the melanoblastic type; (8) the sarcomatous type, and (9) the lipomatous type.

Globus some years later introduced another classification, in which he had six subdivisions, as follows: (1) tumors from primitive meningeal tissue, or meningioma indifferentiale or mesenchymatous meningioma; (2) meningioma omniforme, or primitive meningioma;

16. Elman, R.: Bull. Johns Hopkins Hosp. **34**:99, 1923.
17. Hassin, G. B.: Arch. Neurol. & Psychiat. **23**:65, 1930.
18. Foot, N. C.: Arch. Path. **30**:198, 1940.
19. Bailey, O. T.: (*a*) Arch. Path. **21**:584, 1936; (*b*) **30**:42. 1940.

(3) meningioma pachymeningeale or dural fibroblastoma; (4) meningioma leptomeningeale; (5) meningioma piale, or pial or vascular meningioma, and (6) sarcomatous meningioma. Cushing and Eisenhardt evolved a more complicated classification, in which they arranged subtypes of meningioma.

O. T. Bailey considered these more recent attempts at subdivision of the tumors diagnosed as meningioma into special classes of diverse nature as unfortunate.

There has been some discussion in regard to the terms used to designate the malignant type of tumor. Craig [20] first used the term "malignant meningioma," but Penfield was of the opinion that the tumors thus named belong to the sarcomatous group. He pointed out that ordinary meningioma invades the skull and produces hyperplasia of the bone but that sarcoma of the dura mater destroys the skull. He stated that sarcoma of the dura mater occurs more frequently in the spinal canal but that in almost all instances it has clusters of arachnoid cells, disclosing its origin from the dura and leptomeninges. It recurs locally but does not metastasize; however, in histologic appearance and biologic characteristics it differs strikingly from meningioma, although the two types of neoplasm evidently arise from the same type of cell, just as, elsewhere in the body, fibroma and fibrosarcoma behave differently although both arise from fibroblasts. Opinion is not unanimous as to the nature of the type cell, and until this matter has been settled, I think that the term "malignant meningioma" should continue to be used. This designation expresses clearly that the neoplasm is meningioma and that it is malignant.

In a series of 557 intraspinal neoplasms reported by Rasmussen, Adson and me,[21] 140 (25 per cent) were meningioma. This number was exceeded in this series by neurofibroma only. Of these 140 tumors diagnosed as 23 (16.5 per cent) were cervical, 115 (82 per cent) thoracic and 2 (1.5 per cent) lumbar in location. There is no reasonable explanation for the preponderance of tumors at thoracic spinal sites. Of the 140 intraspinal neoplasms, 130 (93 per cent) were intradural and only 10 (7 per cent) were both intradural and extradural.

Brown [22] studied 130 of these tumors classified as meningioma. He excluded the tumors diagnosed as angioma, because they had been studied separately, and all tumors which did not contain some of the specific cells of meningioma, i. e., the arachnoid cells. He thus excluded tumors which he regarded as pure fibroma, osteoma, osteochondroma, chondroma and lipoma despite a meningeal location, because he felt

20. Craig, W. McK.: Surg., Gynec. & Obst. **45**:760, 1927.

21. Rasmussen, T. B.; Kernohan, J. W., and Adson, A. W.: Ann. Surg. **111**:513, 1940.

22. Brown M. H.: Unpublished data.

that he had not proved that the stromal elements could outgrow and ultimately replace the original neoplastic element, although theoretically this was a possibility. He finally concluded that in the group that was left there were 73 tumors (56 per cent) of the meningothelial type, 28 (22 per cent) of the fibroblastic type, 15 (12 per cent) of the psammomatous type, 4 (3 per cent) of the osteoblastic type, 2 (2 per cent) of the lipomatous type, 1 of the chondromatous and 1 of the melanomatous type. Six tumors were malignant, so that 4.6 per cent of the intraspinal tumors in this group were cytologically malignant. He found that the average duration of symptoms before the patients sought surgical relief in the 6 cases of malignant growth was thirteen months, in contrast to twenty-three months in the series of 130 cases and thirty-six months in those of meningioma of the psammomatous type.

Turner, Craig and I [23] have just completed a study of 36 cases of malignant meningioma encountered in a series of 370 cases of intracranial meningioma, i. e., intracranial meningioma was cytologically malignant in 10 per cent of this group of cases. This should be compared with the 4.6 per cent of cases in which intraspinal meningioma was found malignant by Brown.[22]

Site and Extension of Meningioma and Age of Patients.—As mentioned previously, Rasmussen, Adson and I noted among 140 tumors classified as intraspinal meningioma 10 that were both intradural and extradural. Elsberg [24] encountered several extradural tumors with no apparent subdural attachment. Brown [22] explained that in the cases which he studied the extradural tumor possibly had an attachment to the arachnoid which had disappeared during the process of growth. Arachnoid tufts frequently grow into the dura, and these tufts have arachnoid cell "caps" from which meningioma frequently arises. In such cases meningioma may grow early, and almost totally, into and through the dura and come to lie entirely outside this membrane. It also must be remembered that meningioma derives its abundant blood supply from the dura. Meningioma, whether of the cerebral or of the spinal meninges, does not, as a rule, break through the pia and invade the underlying neural tissue. This occurs more frequently in the brain, but Brown [22] pointed out that malignant meningioma of the spinal canal may break through the pial barrier and invade the spinal cord. Such an occurrence, however, is rare; in most cases meningioma simply compresses and distorts the cord.

Meningioma of the cerebral meninges in many instances invades the skull and produces osteoma—a large growth. A similar phenomenon never occurs in cases of meningioma of the spinal canal because the

23. Turner, O.; Kernohan, J. W., and Craig, W. McK.: Surgery, to be published.

24. Elsberg, C. A.: Surg., Gynec. & Obst. **46**:1, 1928.

epidural space is capacious and the dura does not act as periosteum for the spinal column, in contrast to its close attachment to the skull, for which it acts as periosteum.

Rasmussen, Adson and I noted, and Brown pointed out, that the possibility of meningioma can almost be ignored when considering tumors of the cauda equina.

Meningioma occurs most commonly in persons who are in the third, fourth and fifth decades of life but may occur in children or in elderly patients. Ingraham [25] encountered meningioma in 2 of 16 children who had tumors of the spinal cord; in one the neoplasm was solitary and in the other it was associated with von Recklinghausen's disease. Stookey,[26] in his series of tumors of the spinal cord among children did not find any that could be classified as meningioma. There are few statistical data on the age or sex distribution of tumors of this classification.

Pathologic Characteristics.—Meningioma usually is a small, rounded, nodular tumor attached to the dura; occasionally, it is flat or sessile. When it is removed, the associated dura must be removed with it so that tumor cells will not be left behind; if this procedure is not carried out, recurrence must be expected. Seeding of meningioma from tissue misplaced during operation, with recurrence outside the skull, has been reported by Cushing and Eisenhardt. As a rule, meningioma is extremely vascular, and the vascularity adds to the difficulty of surgical removal.

The histologic picture is characteristic. Large epithelial-like cells usually grow in small islands or lobules; these tend to form whorls, which sometimes are loose and sometimes tight, and have a tendency to degenerate at the center. Precipitation of lime salts and iron in these degenerated regions produces the commonly found, characteristic psammoma bodies. These psammoma bodies usually are small, rounded, concentrically laminated concretions, which usually stain for calcium and iron. Sometimes they are so numerous that a gritty sensation is perceived when the tumor is cut with a knife. Because of this finding, Virchow [27] applied the term "psammoma" to this form of meningioma. Sometimes psammoma may form without whorls in the walls or lumens of blood vessels or in hyalinized tissue. The amount of stroma in meningioma varies markedly; this variation in the amount as well as in the character of the stroma gives neoplasms of this type varied appearances and undoubtedly has added much to the confusion in regard to

25. Ingraham, F. D.: Am. J. Surg. **39**:342, 1938.

26. Stookey, B.: Am. J. Dis. Child. **36**:1184, 1928; Arch. Neurol. & Psychiat. **18**:16, 1927.

27. Virchow, R.: Die krankhaften Geschwülste, Berlin, August Hirschwald, 1863.

their nomenclature and classification. At times the stroma may be so abundant that it almost completely overshadows the neoplastic cells. At times the original neoplasm probably has been replaced completely by the stroma, which may be fibrous tissue, cartilage, bone or even adipose connective tissue.

Rare Forms of Meningioma.—There are several special tumors of meningeal origin which should be mentioned, although they are rare. One of the most spectacular of these is melanoma. Melanin-containing cells in the arachnoid, particularly in that of the medulla and upper portion of the spinal cord, are well known and have been described recently by Taft.[28] Schnitker and Ayer[29] found 30 recorded cases of primary melanoma of the meninges of the brain and spinal cord and reported a case of their own. Ray and Foot[30] reported 2 additional cases; in one the tumor was primary in the meninges of the spinal cord, and in the other it was primary in the meninges of the brain; they considered both of these tumors as comparatively benign. Brown[22] in his series found only 1 example of primary melanoma of the spinal meninges.

Brown,[22] as mentioned earlier, described 4 tumors, diagnosed as osteoblastic meningioma, in which he found zones of formation of mature bone with some small foci of osteoid tissue and hyaline cartilage. He also described 2 lipomatous or lipoblastic specimens of meningioma. One had recurred after surgical removal, and the tissue of the recurrent tumor was identical with that of the original tumor. These tumors contained areas of adipose connective tissue intermingled with typical meningioma cells; Stookey also described intradural lipoma of the spinal cord. Haverfield and Walker[31] described a lipoblastic form of meningioma of the cerebral meninges and pointed out that the presence of fat in a tumor arising from the meninges does not necessarily indicate that the tumor is lipoblastic meningioma. They stressed the point that the cells of meningioma degenerate and that the presence of intracellular fat does not necessarily indicate that the tumor is lipoblastic meningioma. Brown also described a chondromatous type of meningioma in his series of cases of meningioma of the spinal cord. In this tumor, islands of hyaline cartilage were associated with clusters of arachnoid cells.

Another, even rarer neoplasm of the meninges is the diffuse meningiomatous type, such as that described by Weinberger,[32] who differentiated this type of neoplasm from meningeal gliomatous or

28. Taft, A. E.: Arch. Path. **30**:1073, 1940.
29. Schnitker, M. T., and Ayer, D.: J. Nerv. & Ment. Dis. **87**:45, 1938.
30. Ray, B. S., and Foot, N. C.: Arch. Neurol. & Psychiat. **44**:104, 1940.
31. Haverfield, W. T., and Walker, A. E.: Arch. Surg. **42**:371, 1941.
32. Weinberger, L. M.: Am. J. Cancer **38**:1, 1940.

sarcomatosis. Brown and I [33] encountered 1 such neoplasm, and we felt that it represented an embryonal tumor arising in leptomeninges which had been arrested in development.

Still another type of neoplasm is found in the spinal meninges, namely, a tumor metastatic from glioma of the brain to the spinal cord. Russell and Cairns [34] first emphasized the fact that this occurrence is much more frequent than is usually realized. They reported 8 cases in which primary glioma of the brain was associated with a subarachnoid spinal metastasis; these lesions were observed in 8 of 22 cases in which complete necropsy was performed. These metastatic lesions arose from slowly growing, as well as from rapidly growing, glioma. My own observations fully confirm the observations and findings of Russell and Cairns except in regard to the frequency of metastasis to the spinal cord.

It must also be remembered that almost any malignant neoplasm, whether carcinoma or sarcoma, can and does extend or metastasize to the spinal cord or meninges, producing either a local mass or diffuse meningeal carcinomatosis or sarcomatosis.

TUMORS AND MALFORMATIONS OF BLOOD VESSELS INVOLVING THE SPINAL CORD AND ITS MENINGES

The lesions included in this section have no specific location, since they are found extradurally, intradurally, in the pia-arachnoid and in the substance of the spinal cord itself. They also may occur at any level of the spinal cord or meninges. Rasmussen, Adson and I encountered in our series of tumors of the spinal cord 52 that were tumors of blood vessels. Of the extramedullary tumors, 8.5 per cent were of vascular origin; of 64 intramedullary tumors, 7.5 per cent were of vascular origin. In this same series of cases, 10 neoplasms (19 per cent) were cervical, 33 (63 per cent) were thoracic and 9 (17 per cent) were lumbar in location. Twenty-eight (54 per cent) were extradural, 19 (36 per cent) were intradural but extramedullary and 5 (10 per cent) were intramedullary.

Turner and I [35] studied in more detail the same series of cases except 6 that were excluded for several reasons. The classification of blood vessel tumors used by Bailey and Cushing [36] was followed fairly closely, and our series was subdivided into vascular malformations, of which there were 18, and vascular neoplasms, of which there were 28.

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33. Brown, M. H., and Kernohan, J. W.: Arch. Path. **32**:651, 1941.

34. Russell, D. S., and Cairns, H.: J. Path. & Bact. **33**:383, 1930.

35. Turner, O., and Kernohan, J. W.: Arch. Neurol. & Psychiat. **46**:444, 1941.

36. Cushing, H., and Bailey, P.: Tumors Arising from the Blood-Vessels of the Brain: Angiomatous Malformations and Hemangioblastomas, Springfield, Ill., Charles C. Thomas, Publisher, 1928.

The vascular malformations were subdivided into two types, telangiectasis and angioma; the latter sometimes is referred to as hamartoma. The group of lesions caused by telangiectasis was unimportant, since they usually were discovered accidentally and had not produced symptoms. They were dilated, almost cavernous capillaries, and normal glia, nerve or connective tissue was present between the blood spaces. There is also a disease known as hereditary (familial) multiple telangiectasia, occasionally referred to as Osler's disease, which sometimes involves the nervous system.

The angioma (or hamartoma) may be venous or arteriovenous and may simulate a neoplasm; the walls of the vessels frequently are malformed. They may be purely venous and contain venous blood or they may be arteriovenous and contain an admixture of venous and arterial blood. At times the actual arteriovenous communications can be demonstrated at operation. Since these lesions are rarely excised, it was difficult to obtain tissue for histologic study except when found incidentally at necropsy. Such lesions are rare among children in spite of the obvious malformation. Hamartoma usually is composed of a mass of tortuous, interlacing, dilated vessels, which may or may not pulsate, depending on whether arterial or venous components are in excess. If venous components are in excess, pulsations are not present, and the lesion is known as an angioma venosum; if the arterial components are more numerous, pulsations frequently are visible and the tumor is known as arteriovenous angioma. One of the tumors of this type studied by Turner and me involved a nerve root. It was excised, and histologic study revealed that the walls of the blood spaces were poorly formed although some elastic tissue and smooth muscle were present; hence this lesion was an example of arteriovenous angioma. There was no nodular proliferation of the media or the intima and no splitting or duplication of the internal elastic lamina.

Turner and I subdivided the 28 vascular neoplasms as follows:

A. Capillary neoplasms
   1. Capillary hemangioma (2 cases, 7.1 per cent)
   2. Hemangioendothelioma (10 cases, 35 per cent)
   3. Capillary hemangioblastoma (2 cases, 7.1 per cent)

B. Cavernous neoplasms
   1. Cavernous hemangioma (7 cases, 25 per cent)
   2. Cavernous hemangioblastoma (2 cases, 7.1 per cent)

C. Hemangiosarcoma (5 cases, 17.9 per cent)

We found 2 examples of capillary hemangioma. These 2 tumors were composed of dilated capillary spaces with little sign of cellular activity in the thin walls. The capillaries were dilated to such a degree that

with justification we could have used the term "cavernous capillary hemangioma." There were 10 examples of capillary hemangioendothelioma; of these, 3 were extradural, 5 were subdural but extramedullary and 2 were intramedullary. This is the best known and the most interesting of tumors of the blood vessels because Lindau [37] studied and reported on angiomatous cysts of the cerebellum. The syndrome now referred to as Lindau's disease consists of angiomatous cysts of the cerebellum, angioma of the retina, cysts of the pancreas, adenoma or carcinoma of the kidneys and, frequently, other congenital malformations. Russell [38] and others have reported cases in which capillary hemangioma of the spinal cord was associated with syringomyelia. Since the last report by Rasmussen, Adson and me on tumors of the spinal cord, Craig, Wagener and I [39] have encountered a similar case. This observation indicates that the association of hemangioma of the spinal cord and syringomyelia with Lindau's disease is not as rare as has been supposed. I have encountered another case recently.

These neoplasms are a characteristic brick red; on close inspection numerous small holes, which represent the larger blood vessels, can be observed. The histologic picture also is characteristic; it is that of a mass of closely packed capillaries lined with many endothelial cells, some of which are swollen and vesicular and appear to block the lumens of the capillaries. The vesicular cells contain lipoids, as demonstrated by the stain for fats. There are also many cells containing lipoid between the capillaries. The various silver reticulum impregnation methods demonstrate the characteristic relation of the capillaries to each other and to the larger blood vessels, which act as a center from which the capillaries radiate as the spokes of a wheel from the hub. Lindau's disease is congenital and familial, so much so that Lindau himself felt that all patients who have this disease should be sterilized. I recently saw a boy 16 years of age who had Lindau's disease. His father had died at the age of 24 from similar symptoms of brain tumor; 2 uncles, 2 aunts and the paternal grandfather of the patient also had died of the disease. Lindau emphasized that angioma of the retina and polycystic pancreas are pathognomonic for the syndrome, even more so than the angiomatous cysts of the cerebellum. I am of the opinion that capillary hemangioendothelioma of the spinal cord is not a necessary part of Lindau's disease and that most of the tumors of the spinal cord of this type are solitary neoplasms.

In the series of blood vessel tumors reported by Turner and me, there were 2 examples of hemangioblastoma. These 2 tumors were dif-

37. Lindau, A.: Proc. Roy. Soc. Med. **24**:363, 1931.

38. Russell, D. S.: J. Path. & Bact. **35**:103, 1932.

39. Craig, W. McK.; Wagener, H. P., and Kernohan, J. W.: Arch. Neurol. & Psychiat. **46**:36, 1941.

ferent from hemangioendothelioma in that they were more cellular and cytologically active. There were fewer cells containing lipoid; otherwise they could have been classified as hemangioendothelioma. This extra subdivision may be artificial and unnecessary. There were 7 examples of cavernous hemangioendothelioma and 2 of cavernous hemangioblastoma. These subdivisions could be grouped together; the difference was that cavernous hemangioblastoma was more celluiar and that the blood spaces were lined with many more endothelial cells; these cells were larger and contained more chromatin in their nuclei. There was no sharp line of differentiation between these two subdivisions. Of the 9 tumors, 7 were extradural, 1 was intradural but extramedullary and 1 was intramedullary. One tumor was located in the cervical region, and the others were in thoracic locations. Another interesting and important group of blood vessel tumors were 5 actively malignant growths with many mitotic figures, which were diagnosed as hemangiosarcoma. These were both extradural and intradural, and were found in all segments of the spinal cord. Such tumors are important because of their much poorer prognosis and also because in 10 per cent of this series of 52 cases the blood vessel tumors and malformations were actively malignant; when the malformations of the blood vessels were excluded and the tumors alone were considered, approximately 18 per cent were malignant. In spite of the active malignant process, there was no evidence at operation that any of these tumors had spread along the subarachnoid space, nor was there roentgenologic or clinical evidence of metastasis to other organs.

NEUROFIBROMA

The tumors most commonly known as neurofibroma have had many other terms applied to them, such as "neuroma," "neurinoma," "perineural fibroblastoma," "neurilemmoma," "schwannoma," "peripheral glioma" and so forth. Each of these terms when suggested or first introduced by its originator represented an attempt to designate the tissue or specific cell from which the tumor arose. Such a diversity of names and opinions generally indicates that the problem is complicated, that there has been much interest and work on the particular subject and that a solution acceptable by all has not yet been reached.

Virchow, in 1863, was the first to divide neuroma into two types, namely, true and false. He stated that true neuroma contained nerve cells and nerve fibers, while false neuroma arose from the connective tissue of the sheaths of the nerves. He thus was of the opinion that one type of neuroma originated from connective tissue. This opinion was held universally until 1910, when Verocay [40] introduced the term

40. Verocay, J.: Beitr. z. path. Anat. u. z. allg. Path. **48**:1, 1910.

"neurinoma," which means tumor of nerve fibers. Von Recklinghausen,[41] in 1882, described the disease which now bears his name and introduced the term "neurofibromatosis," as he felt that these tumors were chiefly fibrous and arose from the connective tissue of nerve sheaths, particularly from the endoneurium. Mallory agreed and stated that these tumors arose from connective tissue and suggested the term "perineural fibroblastoma"; since that time there have been many excellent studies on this problem, which probably is not yet solved. It is true that most of these studies have been carried out on tumors of peripheral nerves and on the specific tumors of the eighth cranial nerve, yet the structures of all these nerves and the roots of the spinal nerves are similar. Hence the researches are important in consideration of tumors of the nerve roots of the spinal cord.

Frequency and Distribution.—In a series of 557 intraspinal neoplasms, Rasmussen, Adson and I found that 163 (29 per cent) were neurofibroma, which is the most common type of tumor of the spinal cord and of its nerve roots and membranes. Of these 163 tumors, 35 (21 per cent) were cervical, 70 (43 per cent) were thoracic, 55 (34 per cent) were lumbar and 2 were sacral in location. There was 1 case in which tumors of this type were found at multiple levels; however, this was not a case of von Recklinghausen's disease. Most of the tumors classified as neurofibroma were between the dura and the spinal cord. There were 109 tumors (67 per cent) in this location, while 27 (16.5 per cent) were partially intradural and partially extradural. Twenty-seven tumors (16.5 per cent) were extradural. Sometimes portions of these tumors protrude through the intervertebral foramens and produce large tumors outside the vertebral canal. The protrusion outside the vertebral canal may appear in the neck, in the posterior portion of the thoracic cavity or in the lumbar region. I have encountered several cases in which the protruded portion was underneath the skin over the sacrum and extended from a tumor in, or in the region of, the sacral canal.

Neurofibroma arises from a nerve root either inside or outside the dura; in the large majority of instances it arises from the posterior (or sensory) nerve root. This fact should lead to an earlier diagnosis, since the tumor should produce root pain before the spinal cord is damaged seriously. Furthermore, it is much easier to remove a tumor from a posterior than from an anterior nerve root, and there is less danger of injuring the spinal cord. Neurofibroma rarely is found in the substance

41. von Recklinghausen, F.: Ueber die multiplen Fibrome der Haut und ihre Beziehung zu den multiplen Neuromen, Berlin, A. Hirschwald, 1882.

of the spinal cord, although Rasmussen, Adson and I encountered it there in our series of cases of intramedullary tumors.

Neurofibroma of the spinal axis is rare in infancy and childhood. Ingraham, however, in a study of 16 tumors of the spinal cord among children, found that 3 were neurofibroma. In addition, he reviewed the case of a child who had von Recklinghausen's disease. He did not mention neurofibroma as present in this case, but he did mention spinal meningioma as well as tumors of the skin and brain. In reporting a series of tumors of the spinal cord encountered at the Neurologic Institute of New York, Stookey said that 5 per cent occurred among children, but he did not mention neurofibroma. However, he described 2 tumors diagnosed as sarcoma and 1 diagnosed as fibrosarcoma occurring in the extradural tissues. The incidence of neurofibroma among children reported in the literature varies from less than 1 per cent to more than 10 per cent. However, tumors of the spinal cord are rare among children and those diagnosed as neurofibroma are still rarer. Neurofibroma occurs much more commonly in persons in the middle decades of life and less frequently among elderly patients.

Classification.—No attempt has been made to subdivide the tumors grouped as neurofibroma, as these neoplasms do not lend themselves readily to such subdivision. However, there is one method which could be employed, namely, a method for designation of the degree of malignancy. This would be more important were it not possible to remove these tumors completely. Most tumors classified as neurofibroma are slowly growing, although occasionally an actively malignant one is encountered. A study of such neoplasms might give a valuable clue to the type of tissue from which they arise, i. e., whether they should be classified as sarcoma or as malignant glioma.

Histogenesis.—The chief controversy is whether these specific nerve sheath tumors arise from the sheath of Schwann cells, the endoneurium, the perineurium or from both the latter. As has been mentioned, Virchow and his followers were of the opinion that these tumors arose from the connective tissue around the nerve fibers and bundles; Verocay later stated that these tumors were not composed of connective tissue but of nerve fibers. He further stated that these tumors can become modified by such processes as swelling, edema, formation of cysts, hyalinization and an increase in the number of cells which have a polymorphic type of nuclei, and that they can become malignant and form neurinoma sarcomatodes. At times he also found nerve cells as an integral part of these neoplasms. He reasoned that since they all arose from the same mother cell the fibers were nerve fibers. He also pointed out that in cases of multiple tumors there were malformations of nerve and glial cells with formation of tumor.

Lhermitte and Leroux [42] considered tumors of the peripheral nerves as peripheral glioma because the cells appeared different from mesodermal cells and some contained fat which was analogous to myelin. They agreed with those who claimed that these tumors came from Schwann cells and did not contain nerve fibers, as Verocay had stated. Most embryologists agree that Schwann cells are ectodermal in origin and are analogous to glial cells. Other authors have pointed out that degenerating tumor cells can simulate, and be readily mistaken for, nerve cells. I have made the same observation repeatedly, and at times it is necessary to use Nissl's stain or one of the modifications of this stain in order to rule out the presence of nerve cells in degenerating neurofibroma. Mallory, using special staining methods on tumors fixed in Zenker's solution immediately after surgical removal, claimed that these specific tumors of the nerve sheaths were of fibroblastic origin. He pointed out that they produced fibroglia and collagen fibrils in moderate numbers and that some formed elastic fibrils; he introduced the term "perineural fibroblastoma." Penfield [18] adopted the same term and pointed out that the formation of the long hairlike fibers in these tumors was characteristic of the connective tissue about the neurilemma sheaths. Verocay had been misled by these long fibers and thought they were nerve fibers, but Penfield stained them selectively and demonstrated that they were not. Penfield also demonstrated that a large number of collagen fibers can run through these tumors in a tangled mass, and that such fibers are usually slender and uniform throughout their course. He was of the opinion that these tumors originated in endoneurial connective tissue, especially the fibroblastic palisaded portions of some of the tumors in cases of von Recklinghausen's disease.

Some of the tumors classified as neurofibroma tend to undergo cystic degeneration, and in some regions a gelatin-like tissue is formed without actual production of cysts. In such regions loose star-shaped or elongated cells are present, such as are seen in myxomatous tissue. These cells are not astrocytes, since they do not show specific impregnation and do not have vascular attachments. Neuroglial cells are not found in these tumors. The characteristic palisading can be demonstrated easily. The nuclear palisading will be seen in routine stains with hematoxylin and eosin. If the silver staining methods are used, the palisading of the fibrils between the nuclear rows is plainly demonstrated. Penfield pointed out that in the solitary type of neurofibroma, nerve fibers are not present in the tumor but are present in the capsule. Nerve fibers course through the tumors of von Recklinghausen's disease. The latter type of tumors can be considered as true neurofibroma. Penfield agreed

42. Lhermitte, and Leroux, R.: Bull. Assoc. franç. p. l'étude du cancer **9**:112, 1920.

with Mallory's conceptions completely; i. e., that the fibroblast is the type cell of tumors of nerve sheaths and that the cells are not "neuroglial" and not nervous in nature but represent a particular form of connective tissue. The tumors arise from perineurial or endoneurial connective tissue.

Both Mallory and Penfield considered collagen, fibroglial and elastic tissue specific characteristics of fibroblasts. For a time this seemed to be the crux of the argument, since these tumors contain all three types of tissue. Verocay expressed the opinion that neurofibroma arose from Schwann cells, which he considered capable of forming nerve fibers without parent nerve cells. Masson [43] stated that the tumors in question were schwannoma or peripheral glioma but that nerve fibers were not present. Because of the confusion in regard to the origin of Schwann cells, Harrison [44] undertook some transplantation experiments, which have been accepted generally as proving that Schwann cells are separate and distinct entities. When the ganglionic crest and dorsal half of the spinal cord are removed from an embryo, spinal motor roots develop entirely without sheath cells. If the removal is incomplete, portions of spinal ganglions develop, and the nerves of these segments contain sheath cells. Harrison also observed that some medullary cells migrate from the cord by way of the ventral roots and may give rise to some sheath cells although the other sources are removed. Sheath cells do not form motor nerves vicariously in the absence of motor ganglion cells. These experiments demonstrate conclusively that the sheath cells do not have anything to do with the formation of axons. He also stated that sheath cells are not mesodermal and that they should be regarded as the neuroglia of the peripheral nerves.

Rhoads and Van Wagenen [45] applied certain criteria to fibroblastic tissue, which were fulfilled by acoustic neuroma. They found that the structure of the cells, which can be seen with Mallory's stains, is the same in both fibroblastic tissue and acoustic neuroma; collagen is present in both, as demonstrated by the Mallory stains; elastic fibrils are not common in the tumors but are found occasionally, especially in the stroma; fibroglia fibrils are abundant, but they are too delicate to photograph, and reticulum can be demonstrated in the tumors by proper silver impregnation methods. In view of the aforementioned facts Rhoads and Van Wagenen stated that most pathologists would not hesitate to say that the tumors in question are fibroblastic in nature. Bailey and Herrmann [46] studied 2 cases of von Recklinghausen's disease, in each of

43. Masson, P.: Am. J. Path. **8**:367 and 389, 1932.
44. Harrison, R. G.: J. Comp. Neurol. **37**:123, 1924.
45. Rhoads, C. P., and Van Wagenen, W. P.: Am. J. Path. **4**:145, 1928.
46. Bailey, P., and Herrmann, J. D.: Am. J. Path. **15**:1, 1938.

which multiple tumors were present. Some of the tumors, they felt, arose from the perineurium, but a few small tumors originating in the substance of a nerve root did not have any obvious relation to the perineurium. These authors felt that it was impossible to demonstrate by means of present staining methods the cytoplasm and prolongations of the Schwann cells; so the controversy in regard to the origin of neurofibroma cannot be settled on this basis. Both the endoneurium and Schwann cells form interstitial substances which react in identical fashion to special staining methods. Bailey and Herrmann considered the arguments in the controversy on the origin of neurofibroma as inconclusive, although they seemed to think that tumors of this type arose in the perineurium. In conclusion, they stated that the cells of Schwann are believed to play a minor and secondary role in the production of tumors of the nervous system. They preferred the terms "neurinoma" or "neurilemmoma" to "perineural fibroblastoma."

Masson conducted some experiments on the production of schwannoma (peripheral glioma) and compared the tumors with spontaneous schwannoma. He cut a sciatic nerve and observed the proliferation of the Schwann cells in the proximal and the distal segment. When he cut the nerve in two places, a much more marked proliferation of the Schwann cells occurred at both ends of the isolated segment. This proliferation was a mixture of collagen and schwannian cytoplasm, and Masson felt that the latter could be transformed into collagen. He stated that there was marked broadening of the Büngner bands (proliferated Schwann cells) after degeneration of the myelin. He expressed the opinion that the schwannian syncytium constructed the endoneurial collagen framework and that the endoneurial cells occupied it. In writing of the spontaneous schwannoma he stated that there was no difference in the acoustic tumors, those of the spinal cord and those of the peripheral nerves, and that, whether single or multiple, they were all alike. In these tumors there are two types of cells, namely, Antoni [47] type A and Antoni type B. Cells of type A are fasciculated and polarized; those of type B are reticulated and without polarity. The palisading found in these tumors is inconstant but when present is pathognomonic; Masson explained the palisading as a longitudinal division of the nuclei which lie side by side and stated that the palisading simulates the meissnerian nodules, which, in turn, are similar to the Wagner-Meissner corpuscles. He also described the fat-laden cells in these tumors as macrophages of indefinite origin. His conclusion was that the solitary and the multiple tumors of the nerve sheaths arise from Schwann cells. He called them

47. Antoni, N. R. E.: Ueber Rückenmarkstumoren und Neurofibrome; Studien zur pathologischen Anatomie und Embryogenese; mit einem klinischen Anhang. Munich, J. F. Bergmann, 1920.

"schwannomas," and when they occurred on peripheral nerves, he called them "peripheral gliomas." In this he agreed with the other French workers, including Nageotte,[48] Oberling, and Lhermitte and Leroux.

The most recent investigations on this problem are those reported by Murray and Stout [49] and by Tarlov.[50] These workers approached the problem from different points of view and with different methods. Murray and Stout used methods of tissue culture on (1) segments of sciatic nerves obtained from normal and fetal sources, (2) experimentally regenerating nerves and (3) spontaneous tumors of nerve sheaths. They stated that it was possible to identify Schwann cells in tissue culture and to differentiate them from growing fibroblasts. They summarized their work as follows:

> . . . the specific nerve sheath tumor or neurilemmoma is of schwannian origin. This evidence is based on morphological and physiological similarities between the schwannian outgrowth from normal and experimental nerves and the outgrowth from spontaneous tumors. It is inferred that Schwann cells can and do condition the formation of collagen without the intervention of fibroblasts, since these tumors, whose outgrowth is wholly schwannian, contain considerable collagen.

By study of the behavior of the cells in vitro, they were able to offer an explanation of the cystic degeneration seen in these tumors. They also noted that small vacuoles appeared in some of the cells in vitro. It is possible that these were the beginnings of foam cells as seen in many specific tumors of the nerve sheaths. Murray and Stout used the term "neurilemmoma" to designate the specific tumors of the nerve sheaths.

On the other hand, Tarlov used and depended on direct staining methods. He employed the Dockrill modification of Hortega's silver impregnation method on fresh tissue fixed in solution of formaldehyde–urea–potassium iodide and stained with undiluted silver carbonate. He wrote, "This technic, similar to other silver methods, is not entirely reliable, but it gives fair results in a high percentage of trials if the material is fresh and if the staining is done within six to twenty-four hours after fixation." Schwann cells were impregnated, and fibroblasts were not. When these methods were applied to the specific tumors, the cells were not impregnated; therefore he felt that the tumor cells were fibroblasts. He was not able to impregnate any tumor cells; hence he stated that Schwann cells were not present in these tumors. The appearance of the cells and their nuclei was different from that of Schwann cells and simulated that of fibroblasts. Proliferating Schwann cells in a traumatic neuroma can be impregnated. He pointed out that reticulum is a product of fibroblasts and not of Schwann cells and that since specific tumors of nerve

48. Nageotte, J., in Penfield,[1b] vol. 1, pp. 191-239.

49. Murray, M. R., and Stout, A. P.: Am. J. Path. **16**:41, 1940.

50. Tarlov, I. M.: Am. J. Path. **16**:33, 1940.

sheaths contain reticulum the cells are fibroblasts. Tarlov, like Mallory and Penfield, used the term "perineural fibroblastoma" to designate these tumors. He stated that these tumors arose from endoneurium and not from perineurium, as the optic nerve has perineurium but no endoneurium and perineural fibroblastoma had never been described as having arisen from this nerve. These two recent investigations demonstrate how diverse still are the opinions on the origin of these tumors and also how difficult the solution of the problem has proved to be. It is obvious that valid objections can be raised to both methods of investigation and to both points of view, but it is difficult to offer any reasonable suggestions in aid of further investigation or in explanation of some of the unique characteristics of these strange and interesting tumors.

Types.—Neurofibroma may be either solitary or multiple; the multiple type usually is referred to as a specific entity under the title "von Recklinghausen's disease." Von Recklinghausen first noted the frequent association of multiple neuroma with tumors of the skin and named the entity "neurofibromatosis." Since this first description there have been many reports, mostly reports of single cases and some small series of cases. The condition is now recognized as a definite entity. Penfield and Young [2] stated: "The disease seems to reappear in both sexes of successive generations according to the mendelian law. It must therefore be assumed that there is a defect in the germ plasm which leads to the production of tumors of the nerve sheaths, under appropriate stimulation." Multiple neurofibroma as present in cases of von Recklinghausen's disease has been held by some workers to be different from solitary neurofibroma. Penfield [1] and Penfield and Young [2] pointed out that axis-cylinders coursed through the multiple neoplasms and that these were, as a consequence, true neurofibroma. In the solitary tumor, however, the axis-cylinders of the nerve bundle from which the tumor arose were in the capsule of the tumor and not within the tumor itself; as a result, it was perineural fibroblastoma. Masson and others expressed the opinion that these tumors were identical. In my own experience I have had much difficulty in distinguishing these types of tumors. However, I have found that the axis-cylinders are encountered more commonly within the tumors in cases of von Recklinghausen's disease than within the solitary tumors; otherwise the two types of tumors are remarkably similar in histologic structure.

Aside from the multiple tumors of nerve roots and trunks so characteristic of the disease, other findings are common. Multiple tumors frequently are found in the sympathetic nerves, and frequently there are meningeal tumors of varying degrees of rapidity of growth, as well as café au lait spots of the skin. These tumors are usually diagnosed as meningioma, although some may be atypical and simulate neurofibroma.

Not infrequently, glioma is found within the substance of the spinal cord. Penfield and Young found ependymoma in the spinal cord in one of their cases. Parker and I [51] reported a case in which all the characteristics of von Recklinghausen's disease were present and, in addition, 4 tumors of the spinal cord which were diagnosed as gliomas; these tumors were astrocytomatous and cellular ependymomatous types. In addition to the multiple tumors, there was syringomyelia. In cases of von Recklinghausen's disease neurofibroma has a predilection for peripheral nerves, which frequently are thickened diffusely, although tumors may be found also on the nerve roots. Solitary neurofibroma has a greater predilection for the nerve roots, although it also may occur along peripheral nerves.

There is another type of neurofibroma which is interesting as well as important. It usually arises from a nerve root close to an intervertebral foramen outside the dura mater. It compresses the spinal cord within the spinal canal, extends through the intervertebral foramen and either lies among the structures of the neck or, more commonly, extends into the thoracic cavity. It usually is referred to as a "dumbbell tumor" or an "hourglass tumor" of the spine. Heuer,[52] in 1929, found 59 cases of this type of growth reported in the literature and added 3 of his own. Four years later, Naffziger and Brown [53] reported 15 cases of the so-called hourglass tumor, and mentioned some others from the literature. In their series of 15 cases they included cases other than those of neurofibroma, such as 4 of meningioma, 4 of hemangioendothelioma, 1 of osteochondroma, 1 of ganglioneuroma and 1 of carcinoma, as well as 4 cases of neurofibroma. The extraspinal expansions of these tumors varied in form from a small nodule to a mass the size of a baby's head.

Harrington and Craig [54] described a case in which the intrathoracic portion was large as compared with the intraspinal portion. The same year (1934) Harrington [55] described 14 cases of intrathoracic neurofibroma; in only 2 of these was there an intraspinal portion that gave rise to neurologic symptoms. The tumors which I have seen belonging to this group were typical neurofibroma, both the intrathoracic and the intraspinal portion. The intrathoracic portion frequently had undergone degeneration with formation of a large cyst in its center, while the remaining peripheral portion was typical grossly and microscopically of the specific tumors of the nerve sheaths.

Neurofibroma rarely occurs in the substance of the spinal cord. It must be sharply differentiated from polar spongioblastoma, which it

51. Kernohan, J. W., and Parker, H. L.: J. Nerv. & Ment. Dis. **76**:313, 1932.
52. Heuer, G. J.: Arch. Surg. **18**:935, 1929.
53. Naffziger, H. C., and Brown, H. A.: Arch. Neurol. & Psychiat. **29**:561, 1933.
54. Harrington, S. W., and Craig, W. McK.: J. A. M. A. **103**:1702, 1934.
55. Harrington, S. W.: J. Thoracic Surg. **3**:590, 1934.

resembles closely. Polar spongioblastoma does not have palisading of the nuclei and does not contain foam cells. Intramedullary neurofibroma may arise from a small nerve bundle which I have often seen close to or attached to a blood vessel deep in the anterior fissure or in the substance of the spinal cord. The nerve bundle which I have seen accompanies a small artery and frequently lies lateral to the central canal and posteromedial to the anterior horns of the lower thoracic portions of the spinal cord.

Another type, namely, the presacral or postsacral type of neurofibroma, should be mentioned, although it scarcely belongs to the group of tumors of the spinal cord. Presacral neurofibroma belongs to the Mittledorf group of tumors and is identical with neurofibroma elsewhere in the body. Postsacral neurofibroma usually is subcutaneous but may extend out from a tumor within the sacral canal or may be primary in a nerve root in the postsacral region.

Pathologic Characteristics.—The tumors classified as neurofibroma vary from 1 to 2 mm. to many centimeters in diameter. The smallest ones usually are found accidentally at necropsy and are subclinical in that they do not produce symptoms; the largest ones are extraspinal extensions. Most of the intraspinal ones are attached to nerve roots, are rounded, are usually 1 to 1.5 cm. in diameter and are elongated or sausage shaped and measure 2 to 3 cm. or more in length. They are encapsulated and have smooth surfaces, and sometimes small nodules are present. They are grayish yellow and usually are firm. The cut surface is grayish, grayish yellow or sometimes yellow; frequently, small cysts and sometimes small brownish red regions representing former small hemorrhages are present. The nerve root usually is attached to one side of the tumor and, as a rule, is not incorporated in the new growth and sometimes is not even firmly attached to the capsule. In the tumor in cases of von Recklinghausen's disease, the nerve frequently enters at one end and emerges from the other. The larger tumors usually have cystic parts, which vary in size; in some tumors, gelatinous regions without formation of cysts are found. These tumors produce symptoms by compressing or distorting a nerve root, which causes pain, or by compressing the spinal cord, which causes partial or complete paraplegia. The two last-named symptoms usually are dependent for the most part on the size of the tumor.

Microscopically, these tumors have a characteristic appearance. Four histologic findings are pathognomonic of neurofibroma, namely, (1) palisading nuclei, (2) interlacing bundles of fibers, (3) small pigmented foci of degeneration and (4) foam cells. These four features usually are present, but sometimes one or more are absent. The palisading of the nuclei is the most characteristic feature and is almost pathognomonic

of this type of tumor. However, it is the least constant of the findings. The nuclei lie side by side in rows like fence posts, and between the rows of nuclei the fibrils of the cells also are parallel. These fibrils can be demonstrated clearly by silver impregnation methods. The most constant and least significant finding is the streaming or interlacing of bundles of cells and fibers with the nuclei. In these bundles the cells and their processes lie parallel. These cells usually are referred to as the Antoni type A because of their fibrillar and polar arrangement. The Antoni type B cell has a loose, reticular, apolar arrangement and is present as the background in all these tumors. Foci of degeneration, even progressing to formation of cysts, are common in these tumors and the pigment, frequently present, is hemosiderin from small hemorrhages which have occurred in these neoplasms.

Some pathologists place much emphasis on the foam cells in arriving at a diagnosis of neurofibroma. These foam cells are laden with fat and have the appearance of xanthoma cells and not of adipose connective tissue. Frequently, the lipoid-containing cells are so numerous as to change the appearance of the tumors, making them yellowish instead of gray. The lipoid is demonstrated easily with any of the usual fat stains. These cells are considered as phagocytes by most pathologists. However, Berwald and I [56] studied a series of cases of neurofibroma in order to determine, if possible, the origin of these foam cells. We felt that we could trace them from their beginnings, and we were of the opinion that they were not phagocytes but were degenerated tumor cells which could be seen in all stages of their development. Murray and Stout, with tissue cultures of neurofibroma, saw vacuoles in some of the growing cells in vitro, especially in cells of the older cultures. It is possible that these vacuoles were similar to the earliest formation of foam cells as seen by Berwald and me.

There is commonly found in neurofibroma some variation in the size of the nuclei. Frequently, large or almost giant nuclei are seen, and I have found considerable difficulty in deciding whether their large size is the result of increased cell activity and growth or a degenerative phenomenon. The evidence seems to favor a degenerative process. Mitotic figures are uncommon, and frank malignant processes are rare, in neurofibroma of the spinal cord. If a genuine malignant process is present, it usually is found in the extraspinal extension of the so-called dumbbell tumor, although even this form is rarely malignant. When the dumbbell tumor is cytologically malignant, it has more the appearance of sarcoma than that of glioma. Metastasis from intraspinal neurofibroma is almost unheard of.

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56. Berwald, W. P. E., and Kernohan, J. W.: Unpublished data.

Stout [57] and later Lanford and Cohn [58] described specific tumors of peripheral nerves which simulate medulloepithelioma or medulloblastoma and are sometimes referred to as neuroepithelioma. Stout expressed the opinion that these tumors come from rests carried from the neural tube during the migration of the tissues to form peripheral nerves. Penfield expressed the opinion that these tumors represent schwannoma. None of these tumors have been found or described as having occurred within the vertebral canal.

Comment.—At times it is difficult to decide whether the condition in a specific case should be classified as von Recklinghausen's disease. If a patient has two, three or more tumors diagnosed as neurofibroma and none of the other stigmas of von Recklinghausen's disease, it is questionable whether the condition should be classed as von Recklinghausen's disease. I have been in the habit of classifying such cases as instances of the solitary variety of neurofibroma, although this decision is open to criticism. At times a correct interpretation is important because of the hereditary traits of von Recklinghausen's neurofibromatosis.

INTRAMEDULLARY TUMORS

Neoplasm that arises in the substance of the spinal cord is considered rare, yet Rasmussen, Adson and I found that in about 17 per cent of all cases of tumor of the spinal canal in our series the growth was in the substance of the spinal cord or the filum terminale. Since this report was published, I have seen 16 additional cases, and the ratio has become still higher. These figures are based on cases in which the character of the tumor was microscopically verified. During the period covered by this study, however, many more tumors were seen at surgical explorations at the Mayo Clinic which could not be excised or submitted to biopsy. This would indicate that intramedullary tumors are really more common than usually thought.

Classification.—Prior to the studies of Bailey and Cushing [59] little serious or successful effort had been expended on any classification of the tumors of the brain grouped as glioma. The classification proposed by Bailey and Cushing has now been accepted by almost all pathologists and has been modified only slightly. Prior to 1931 insufficient material had been collected by any one group to justify an attempt at classification of intramedullary or primary tumors of the spinal cord. At that time

57. Stout, A. P.: Proc. New York Path. Soc. **18**:2, 1918.

58. Lanford, J., and Cohn, I., cited by Penfield.[1b]

59. Bailey, P., and Cushing, H.: A Classification of the Tumors of the Glioma Group on a Histogenetic Basis with a Correlated Study of Prognosis, Philadelphia, J. B. Lippincott Company, 1926.

Woltman, Adson and I [60] reviewed 51 intramedullary tumors of the spinal cord and found them similar in type to glioma of the brain. However, a marked difference in the distribution of the forms of glioma was found. This variation is expected if the normal histologic structure of the spinal cord, conus medullaris and filum terminale is considered. A large preponderance of astrocytes is present in the brain, and the majority of tumors of the brain are either astrocytoma or the more rapidly growing varieties of astrocytoma, namely, glioblastoma and spongioblastoma multiforme. The ependymal cells that line the ventricular system of the brain are comparatively few, and ependymoma of the brain is not common. Astrocytes are less numerous in the spinal cord than in the brain, and astrocytoma is proportionately less common. Ependymoma is much more common in the spinal cord. The central canal is patent at birth but gradually becomes obliterated, and the ependymal cells are distributed irregularly throughout the gray commissure in the large majority of cases, as Brown [61] recently pointed out. These cells when displaced from their normal position and usual environment may have a greater predilection for neoplasia than other normal cells of the spinal cord. However, identical cells are present in the spinal cord and brain, and it is expected that similar tumors will be found in these two structures. There is a large collection of ependymal cells in the conus medullaris, with fewer glial and nerve cells. This excess of ependymal cells is the result of the presence of the ventriculus terminalis. The growth and structure of this have been described by me.[62] In this region ependymoma is found much more frequently than any other type of glioma. The spinal cord continues as a small attenuated filament downward within the cauda equina, as the filum terminale. The normal structure of this filament was described by Harmeier,[63] who demonstrated that it contained all the cell elements present in the spinal cord but that the ependymal cells were in excess. These cells do not have a regular or characteristic arrangement but are scattered irregularly as islands or small tubules throughout the filum terminale, at times lying close to the pia mater.

Frequency and Distribution.—Rasmussen, Adson and I found that of 557 tumors of the spinal cord, 64 were intramedullary. Of these, 19 (30 per cent) were cervical, 39 (61 per cent) thoracic and 6 (9 per cent) lumbar in location. We also found 32 examples of glioma of the filum terminale and conus medullaris. There was a greater proportion of

60. Kernohan, J. W.; Woltman, H. W., and Adson, A. W.: Arch. Neurol. & Psychiat. **25**:679, 1931.

61. Brown, R. W.: The Central Canal of the Spinal Cord, Thesis, University of Minnesota Graduate School, 1940.

62. Kernohan, J. W.: J. Comp. Neurol. **38**:107, 1924.

63. Harmeier, J. W.: Arch. Neurol. & Psychiat. **29**:308, 1933.

tumors classified as ependymoma than Woltman, Adson and I [64] reported earlier. Rasmussen, Adson and I found that of the 64 intramedullary tumors, 33 (51 per cent) were ependymoma or ependymoblastoma. If the tumors which arose from the filum terminale were included, the tumors diagnosed as ependymoma constituted 67.7 per cent of the entire series of 96 tumors. This is an extremely high preponderance for any one type of tumor, especially a type which is considered rare. A larger series might readily change this percentage, but since these figures were published, most of the intramedullary tumors and all of the tumors diagnosed as glioma of the filum terminale which I have examined have been ependymoma, so that at the moment even a higher percentage of primary tumors of the spinal cord and filum terminale are ependymoma.

In addition to the tumors classified as ependymoma, the 64 intramedullary tumors included 10 tumors (15.5 per cent) that were diagnosed as astrocytoma and polar spongioblastoma; the latter type now is included with astrocytoma. Other tumors in the group were diagnosed as follows: 3 (5 per cent) as oligodendroglioma and oligodendroblastoma, 3 as glioblastoma multiforme, 3 as medulloblastoma, 2 as tumors of the nerve cells, 5 (7 per cent) as hemangioendothelioma, 3 as primary melanoepithelioma, 1 as fibrolipoma and 1 as neurofibroma.

Rasmussen, Adson and I did not discuss the detailed histologic observations on these intramedullary tumors nor did we extensively review the literature. However, Foerster and Bailey,[65] in a recent review of the literature on the subject, easily found references to almost 100 such tumors and reported 7 of their own; of these 7 tumors, only 2 were ependymoblastoma, while 4 were astrocytoma; of the latter, 1 was fibrous, 2 were protoplasmic and 1 was both fibrous and protoplasmic. The seventh tumor was glioblastoma multiforme. In their review of the literature they found that more than 50 per cent of the tumors were ependymoma, which corresponded closely to the percentage reported by Rasmussen, Adson and me. Foerster and Bailey found that 15 per cent of the tumors in the reported cases belonged to the group of glioblastoma multiforme. They pointed out that glioma of the spinal cord is not encapsulated and thus is similar to glioma of the brain. Glioma of the spinal cord sometimes infiltrates the substance but sometimes is surrounded by a zone of gliosis of varying thickness. Glioma frequently is found in the inner portions of the posterior columns, where syringomyelia is found most commonly. Foerster and

64. Kernohan, J. W.; Woltman, H. W., and Adson, A. W.: Arch. Neurol. & Psychiat. **29**:287, 1933.

65. Foerster, O., and Bailey, P.: A Contribution to the Study of Gliomas of the Spinal Cord with Special Reference to Their Operability, in Jubilee Volume for Davidenkov, Leningrad, State Institute for the Publication of Biologic and Medical Literature, 1936, pp. 9-67.

Bailey further pointed to this association as a possible causal relationship between glioma and the method of formation of the posterior septum of the spinal cord.

The largest series of histologically verified tumors of which adequate microscopic descriptions are available is the earlier group described by Woltman, Adson and me. Except for summaries, the report contains few clinical data. However, from a pathologic point of view the report is a fairly complete summary and review.

Ependymoma.—Woltman, Adson and I found in our series 21 tumors diagnosed as ependymoma; these were classed as of three types. Those of the first type were designated as the epithelial group and were characterized by canals lined with ependymal cells simulating the cellular arrangement of the central canal of the spinal cord. Tumors of the second type were designated as the myxopapillary group, in which the arrangement of ependymal cells simulated the choroid plexus except that the cells did not contain the mucus common in tumors of the choroid plexus and the stroma of the papillae presented myxomatous degeneration. This sometimes had proceeded to an advanced degree.

The tumors comprising the third class were known as the cellular type. This term was somewhat unfortunate, since it seems to imply cellular activity. However, the term was meant to designate a fairly cellular and slowly growing neoplasm. The cells were not arranged in any particular pattern except occasionally around blood vessels. There were small regions free of nuclei but containing cell processes, which have been referred to as *ballons* and are common in this particular type of ependymoma. Fletcher-Kernohan and I [66] found that the centers of some of these *ballons* contained small concretions which stained with eosin and which had an affinity for mucicarmine. The nuclei of the cells of these tumors were larger than those of astrocytes, with which they might be confused, and it was noted that the nuclear chromatin was collected into larger granules. The cells did not have intracellular neuroglial fibrils, but heavy cell processes frequently were present, which ended abruptly with a structure resembling a fishtail. In some cells vascular projections were present, but well formed vascular feet were absent. Pseudorosettes were not uncommon. The cells and nuclei were uniform, and mitotic figures or other signs of malignancy were not seen. This type of ependymoma could be differentiated readily from astrocytoma by the use of Cajal's gold chloride and sublimate impregnation method. Astrocytes have a strong affinity for this stain; ependymal cells have no affinity for the gold salts.

66. Kernohan, J. W., and Fletcher-Kernohan, E. M.: A. Research Nerv. & Ment. Dis., Proc. (1935) **16**:182, 1937.

Woltman, Adson and I pointed out that ependymoma has a long preoperative history and that prognosis is favorable after removal of the tumor. Even after partial removal, prolonged relief frequently follows. Some years later, Fletcher-Kernohan and I in a review of a large series of cases of ependymoma of the central nervous system, confirmed these findings. The same three subtypes were used for classification of the cases, and it was pointed out that this subdivision was useful for histologic purposes. It is unusual to find any one neoplasm in which only one of the three types is represented, as many of the specimens contain two and sometimes all three types. It was intimated that ependymoma responds fairly satisfactorily to roentgen therapy; in fact, the response has been better than the comparative benignancy of the lesions would indicate. Rasmussen, Adson and I included some cases of ependymoblastoma in our group of cases of ependymoma. Ependymoblastoma is at times malignant, with cells larger than those of ependymoma; there is also considerable variation in the size of the nuclei, in the number of mitotic figures, which at times may be numerous, and in the numbers of hyperchromatic nuclei and so forth. I have seen one tumor of this type spread rapidly and widely up and down the subarachnoid space. Roentgen therapy has not seemed to affect these tumors nearly as effectively as the more slowly growing neoplasms. Horrax and Henderson [67] removed at two stages a tumor diagnosed as ependymoma, which involved the entire length of the spinal cord; it measured 38.5 cm. in length.

Oligodendroglioma.—An observation which Fletcher-Kernohan and I made and which I have confirmed consistently since is the close association between so-called cellular ependymoma and oligodendroglioma. In many of the tumors classified as the cellular type of ependymoma there were areas which had a honeycomb appearance; Bailey and Cushing demonstrated that this appearance is characteristic of oligodendroglioma. After numerous attempts, I stained several specimens, using the silver carbonate method of Hortega; this stain is fairly specific for oligodendroglial cells; these preparations show immature oligodendroglial cells in considerable numbers. Fletcher-Kernohan and I were led to believe that oligodendroglioma and ependymoma have a common origin in spite of the fact that these observations do not conform to the accepted embryologic origin of these cells. Hortega [68] stated that indifferent cells differentiate into oligodendrocytes. As a result of these observations, Fletcher-Kernohan and I suggested a slight modification of the standard accepted classification of tumors in the group known as glioma as originally evolved by Bailey and Cushing. Rasmussen, Adson

67. Horrax, G., and Henderson, D. G.: Tr. Am. Neurol. A. **64**:172, 1938.
68. del Rió Hortega, P.: Bol. real. Soc. espafi. d. hist. nat. **21**:63, 1921.

and I found only 3 tumors diagnosed as oligodendroglioma in our group of 64 intramedullary tumors, and Woltman, Adson and I found 2 in our earlier series of 51 neoplasms. One of these 2 had extended outside the spinal cord and spread up and down the subarachnoid space, producing a gliomatous meningitis around the spinal cord.

In a high percentage of cases of oligodendroglioma of the brain the tumor contains small masses of calcium, sometimes in sufficient amount to be visualized on roentgenologic examination, but in the 3 cases of oligodendroglioma of the spinal cord no mention was made of calcium. I have been unable to observe calcium on repeated microscopic examination. The tumors have a characteristic appearance; the nuclei are small and stain intensely with hematoxylin, so that they simulate the nuclei of lymphocytes; a clear zone having the appearance of a halo is around each nucleus, and this zone, in turn, is outlined by the sharp cell membrane. The cells lie close together and a section of any of these tumors has a honeycomb appearance.

Astrocytoma.—The second most common type of glioma of the spinal cord is astrocytoma. Rasmussen, Adson and I encountered 10 tumors of this type in our series. Polar spongioblastoma was included with astrocytoma in accordance with the findings of Russell, who demonstrated by tissue culture methods that polar spongioblasts were in reality astrocytes. The fact that such tumors grow in a restricted region in all probability alters the structure and even some of the characteristics of the cell processes. At times this alteration makes diagnosis difficult. Astrocytoma has a fairly typical appearance, with the neuroglial fibrils seen after use of Mallory's phosphotungstic acid–hematoxylin stain, the vascular processes and the affinity of the cells for Cajal's gold chloride and sublimate impregnation. At times it is necessary to differentiate astrocytoma from the cellular type of ependymoma. Tumors of the latter type have no affinity for the Cajal gold salts, and intracellular fibrils are not seen with the aid of the Mallory stain. However, ependymal cells as seen in ependymoma have processes which are broad and not like those of neuroglia. One of the tumors diagnosed as astrocytoma in Foerster and Bailey's series was of mixed protoplasmic and fibrous type; this tumor was 25 cm. in length.

Spongioblastoma Multiforme.—Glioblastoma or spongioblastoma multiforme is the most common type of glioma of the cerebrum, although it is one of the least common tumors of the spinal cord. Foerster and Bailey found 15 such tumors in a review of 100 intramedullary tumors of the spinal cord described in the literature. Rasmussen, Adson and I found only 3 examples in our series. One of these 3 tumors was identical with tumors of similar type seen in the cerebrum; there was marked variation in the size and shape of the nuclei and cells, and there were

tumor giant cells, numerous mitotic figures, areas of necrosis and degeneration, and proliferation of the endothelial cells lining and surrounding the blood vessels. The other tumors were less characteristic but were considered spongioblastoma (glioblastoma) multiforme by Woltman, Adson and me, who thought them to be a slowly growing type of this tumor. Globus and Strauss [69] in their original description of spongioblastoma multiforme reported one in the spinal cord. It is interesting that more than 30 per cent of the tumors of the brain diagnosed as glioma should belong to this group and that only 5 per cent of intramedullary tumors of the spinal cord should be of this group.

Miscellaneous Lesions.—In addition to the aforementioned types, Rasmussen, Adson and I found the following types represented in our series: medulloblastoma, 3; ganglioneuroma, 2; hemangioendothelioma, 5; primary melanoepithelioma, 3; fibrolipoma, 1, and neurofibroma, 1. As a rule, medulloblastoma occurs in the midline of the cerebellum and in children. The 3 tumors of this type mentioned were not associated with medulloblastoma of the cerebellum. This is worthy of note, because cerebellar medulloblastoma sometimes becomes implanted on the nerve roots or invades the substance of the spinal cord, so it would seem that on occasion it could arise in the spinal medulla. Because of the large numbers of nerve cells in the spinal cord, ganglioneuroma could be expected more frequently. Hemangioendothelioma has been mentioned in conjunction with blood vessel tumors of the spinal cord. Melanoepithelioma probably is not primary in the substance of the spinal cord but more likely arises from the meninges. Since necropsy was not performed in any of the 3 cases reported by Rasmussen, Adson and me, the possibility that the tumors were secondary to some other focus must be considered. However, these 3 cases, along with others, were clinically investigated by Moersch, Love and me [70]; evidence of another, primary tumor could not be found. Melanoma has been found by others to be primary in the meninges, and this fact is now generally accepted. Woltman, Adson and I reported 1 lipoma in the substance of the cervical segment of the spinal cord. At the time of that report 7 or 8 other cases of primary lipoma had already been described in the literature, for example, those of Sachs and Fincher,[71] who reviewed the literature.

Teratoma has been encountered by many writers, especially among children and young people. Masten [72] described a tumor of this type in the sixth cervical segment of the spinal cord of a girl 5 years of age. Ingraham found 3 such tumors in a series of 16 intraspinal tumors of

69. Globus, J. H., and Strauss, I.: Arch. Neurol. & Psychiat. **14**:139, 1925.

70. Moersch, F. P.; Love, J. G., and Kernohan, J. W.: J. A. M. A. **115**: 2148, 1940.

71. Sachs, E., and Fincher, E. F., Jr.: Arch. Surg. **17**:829, 1928.

72. Masten, M. G.: Arch. Path. **30**:755, 1940.

children. Kubie and Fulton [73] reported a teratoma in a child 2 years of age, but this tumor was between the arachnoid and the dura mater and was not intramedullary.

Another group of intramedullary tumors of which little has been written in recent years but which was mentioned more frequently in the older literature comprises the tumors referred to as neuroepithelioma. Penfield and later Woltman, Adson and I pointed out that most of these tumors were ependymoma. Foerster and Bailey stated that neuroepithelioma should in most instances be classified with ependymoma, i. e., ependymoma characterized by small canals. They also said that occasionally neuroepithelioma, like glioma in the retina of the eye, may be found in the brain or spinal cord. Such a case was described by Hartwell and Stevenson [74]; the tumor was found to be extramedullary but in the subarachnoid space. In addition to the neuroepithelial cells, there were some spaces lined with ependymal-like cells.

Another rare condition which should be mentioned is gliomatous meningitis. In most of the cases I have encountered, the tumor arose from some focus or tumor in the brain and spread like a sheet of neoplastic tissue throughout the subarachnoid space. Sometimes it was almost impossible to find the focus. I studied 1 case in which the tumor had extended from the substance of the spinal cord and spread up and down the subarachnoid space. It is remarkable that this does not occur more frequently. O. T. Bailey,[19a] in discussing the relation of glioma of the leptomeninges to neuroglial nests, reported 1 case of extracerebral astrocytoma and reported several cases of extramedullary glioma he had found in the literature. He expressed the opinion that implants from cerebral glioma in the subarachnoid space around the spinal cord could be confused with neuroglial nests, especially in referring to implants from astrocytoma of the brain.

Another group of tumors interesting from the clinical, surgical and histologic points of view are those arising from the filum terminale. Woltman, Adson and I reviewed a series of 25 such tumors. In this series, ependymoma was proportionately more frequent than in the group of tumors of the spinal cord. Seventeen (68 per cent) of the tumors diagnosed as glioma arising from the filum terminale were ependymoma; of these, 9 were cellular and 8 myxopapillary in type. There were none in which canals had formed. We also found 1 tumor, diagnosed as astrocytoma, in which more protoplasmic than fibrillar astrocytes were present; this mixture of two types of astrocytes is uncommon even in the brain. We also described 3 examples of astroblastoma and 2 examples of spongioblastoma (glioblastoma) multiforme.

73. Kubie, L. S., and Fulton, J. F.: Surg., Gynec. & Obst. **47**:297, 1928.
74. Hartwell, J. A., and Stevenson, L. D.: Ann. Surg. **81**:413, 1925.

One of the latter neoplasms was typical and highly malignant; the other was much more slowly growing in type. There were also an excellent example of oligodendroglioma and 1 tumor which we did not classify.

A few cases of the rare extramedullary glioma have been described in the literature. Thompson,[75] in discussing 3 cases of leptomeningioma of the spinal cord, described a tumor which he designated as gliosarcoma. This neoplasm contained some astrocytes and an abundant network of sharply staining fibrils which stained specifically as neuroglial fibrils with Mallory's phosphotungstic acid–hematoxylin. However, since some collagen and elastic tissue were present, he designated it as gliosarcoma. Woltman, Adson and I, in our series of cases, reported 3 cases in which glioma had been removed from the subarachnoid space. In each instance the growth had been attached to the pia and the origin could not be traced to the substance of the spinal cord. It could not be ascertained that the growth did not originate in the spinal cord, as it may have been attached to it by a small pedicle which was overlooked during removal. The patients recovered and further investigation was not possible. We stated that glioma may originate in the subarachnoid space, as we had observed several cases of heterotopic masses of glial or ependymal tissue in the subarachnoid space and postulated that it was possible for neoplasms to originate from such masses.

Another and larger group of neurogenic tumors, most of which could and probably did arise from heterotopic neural elements, are those arising from the sacrum. Adson, Moersch and I [76] reviewed a series of these neoplasms recently and found that some were postsacral and that others were in the bones of the sacrum; the largest number, however, were presacral but posterior to the rectum. Since these neoplasms do not arise from the spinal cord or its coverings, the types will be enumerated only briefly. There were 2 examples of ganglioneuroma, 7 of ependymoma, 1 of neurofibroma and a large series of examples of chordoma, which will be discussed later. The tumors diagnosed as ependymoma did not differ histologically in any way from those found in the spinal cord.

In the voluminous literature now available on von Recklinghausen's disease, many intramedullary tumors have been described as associated with the syndrome. Frequently two or more types of glioma in the spinal cord, as well as syringomyelia, have been found associated with von Recklinghausen's disease. The studies usually have been reports of single cases or of small series of cases. As the tumors are similar to those described in preceding paragraphs, further discussion is not

75. Thompson, T.: Lancet **1**:325, 1929.

76. Adson, A. W.; Moersch, F. P., and Kernohan, J. W.: Tr. Am. Neurol. A. **64**:177, 1938.

necessary except to point out that in all probability every such tumor is the result of a congenital defect.

Intramedullary hemangioblastoma may occur in the spinal cord in the course of Lindau-von Hippel disease. This has been mentioned in discussion of tumors of vascular origin. Intramedullary hemangioblastoma may be associated with syringomyelia.

Syringomyelia.—Since syringomyelia is frequently associated with intramedullary tumors, it should be discussed briefly, although it generally is not considered a neoplasm. The literature on syringomyelia is extensive; Schlesinger [77] listed 1,175 references in his monograph, published in 1902. Jonesco-Sisesti [78] considered intramedullary tumors intimately associated with syringomyelia in certain cases, but in a consideration of the various theories as to the origin of the disease process he stated that syringomyelia may be attributable to a tumor with necrobiosis of the center and prolongations of the cavitation above and below the neoplastic process, a tumor arising from cell rests of embryonic origin or from delayed development of heterotopic cells which had penetrated into the cord during intrauterine life. The prolongations of the cavity are always neuroglial, irrespective of the type of tumor. The tumor may be fibroglioma, ependymoglioma or a more complex type, such as teratoma. The possibility that syringomyelia is a result of degeneration should be considered, but Jonesco-Sisesti was not certain whether the gliosis was the cause or the result of the degeneration. He said that the cavities rarely communicated with the central canal of the spinal cord. He quoted Bielschowsky and Unger [79] in regard to the coexistence of other congenital anomalies. He also pointed out that the association of an intramedullary tumor and a syringomyelic process may be fortuitous, i. e., two separate and distinct lesions, which also may have a common origin and cause. He quoted Leyden,[80] who stated that the condition was congenital and due to incomplete occlusion of the primitive fold situated in the posterior portion of the spinal cord. Jonesco-Sisesti pointed out that Kahler and Pick [80] expressed the opinion that this arrest in development was attributable to a chronic inflammation. He also considered the possibility that syringomyelia was the result of myelitis around the ependyma of the central canal, with softening and cavity formation.

Foerster and Bailey pointed out that glioma may be infiltrative or surrounded by a variable region of gliosis. This occurs in the inner

77. Schlesinger, H.: Die Syringomyelie. Eine Monographie: I, Leipzig, Franz Deuticke, 1902.

78. Jonesco-Sisesti, N.: Tumeurs médullaires associés à un processe syringomyélique, Paris, Masson & Cie, 1929.

79. Bielschowsky, M., and Unger, E.: J. f. Psychol. u. Neurol. **25**:173, 1920.

80. Cited by Jonesco-Sisesti.[78]

part of the posterior columns, where syringomyelia and gliosis occur, and they pointed to this fact as indicating a possible causative relationship between glioma and the method of formation of the posterior septum of the spinal cord. They stated that syringomyelia may arise from many causes, but that there could be no question that in most instances it arose from the breaking down of congenital gliosis, which, in turn, is attributable to malformation of the dorsal part of the spinal cord. In this they agreed with Bielschowsky and Unger, who first postulated this theory of the genesis of syringomyelia. Russell, in discussing a case in which syringomyelia was associated with capillary hemangioma of the spinal cord, referred to 3 other intramedullary tumors which she had examined; 2 of these neoplasms were ependymoma and were associated with syringomyelia; the third tumor, diagnosed as spongioblastoma multiforme, was not associated with syringomyelia. Woltman, Adson and I found syringomyelia associated in 5 of 9 cases of intramedullary tumor in which necropsy had been performed. This is much too high an incidence of the association of a tumor with syringomyelia to be fortuitous. The association was not limited to one kind of tumor, since medulloblastoma was present in 1 instance, oligodendroglioma in 1, ependymoma in 2 and hemangioma in 1.

It is necessary to distinguish between syringomyelia and hydromyelia. The latter is simply a dilatation of the central canal of the spinal cord and is probably congenital. It may occur alone, but it is almost always found in the presence of spina bifida. It practically never produces symptoms, and the cavity seldom is surrounded by gliosis and is always, at least in part, lined by ependymal cells. Occasionally, among some of the major anomalies of the spinal cord associated with spina bifida, associated hydromyelia and syringomyelia are found. These anomalies usually occur among young children and frequently are associated with other malformations which are incompatible with life, or else the spina bifida ruptures or becomes infected, with meningitis as a consequence. Syringomyelia usually is posterior to the central canal. Ependymal cells of the obliterated central canal usually can be found anterior to, or incorporated in, the dense gliosis of the syringomyelic cavity near the anterior fissure. In cases of hydromyelia the pia is usually normal unless acute meningitis has been present, while in cases of syringomyelia the pia invariably is thickened and fibrosed.

The histologic appearance of the wall of a syringomyelic cavity is typical. A dense mesh of neuroglial fibrils is present, as seen with the aid of the Mallory phosphotungstic acid–hematoxylin stain. In cases of syringomyelia unaccompanied with intramedullary tumor there is frequently a replacement of part of the neuroglia by connective tissue, as demonstrated by the Van Gieson stain. This replacement fibrosis is not as a rule so marked in syringomyelia associated with intramedullary

neoplasms. Frequently, there are thickening and hyalinization of the walls of the small arteries in the gliosis, as well as narrowing of the lumens and increase of the connective tissue of the adventitia. Also, there is usually more than one cavity. I have seen as many as six separate cavities at one level; this multiplicity of cavitation can be seen grossly. It is not common to find one cavity extending the entire length of the syrinx; usually, numerous complete or partial septums divide the seemingly large cavity into many small ones.

MISCELLANEOUS TUMORS

Chordoma.—It is not customary to include chordoma when considering tumors of the spinal cord. However, Rasmussen, Adson and I reported 23 tumors of this type, which was 4 per cent of all neoplasms of the spinal cord in our series. Chordoma usually is situated outside the dura, although occasionally it is found to have penetrated this structure, but rarely as far as the arachnoid. Chordoma originates in remnants or rests of the embryonic notochord, which frequently persist for many years after birth in the centers of nuclei pulposi of intervertebral disks. Trauma is supposed to play an important role in the production of some of the neoplasms diagnosed as chordoma. Chordoma is found predominantly in two locations, either in the sacrococcygeal region or in the basisphenoid (the so-called clivus blumenbachii), although it has been described at other levels of the spinal column. Rasmussen, Adson and I found it in the cervical region in 3 instances (13 per cent), in the thoracic region in 2 (9 per cent) and in the lumbar segments of the spinal column in 1 (4 per cent); in 17 cases (74 per cent) it arose from the sacrum.

Although chordoma is not a common neoplasm, it has been recognized and reported in the medical literature for many years. That it is of notochordal origin was first suggested by Müller [81] and later proved by Ribbert.[82] Owen, Hershey and Gurdjian [83] described a case in which chordoma had originated in the cervical portion of the spinal column and found 7 others in the literature. Chordoma is even less common in the thoracic and lumbar portions of the spinal column; however, Hutton and Young [84] described a tumor of this type which originated at the level of the fourth, fifth and sixth thoracic vertebrae. Davison and Weil,[85] and later Zollinger,[86] described chordoma in the lumbar

81. Müller, H., cited by Stewart and Morin.[89]
82. Ribbert: Verhandl. d. Kong. f. inn. Med. **13**:455, 1895.
83. Owen, C. I.; Hershey, L. N., and Gurdjian, E. S.: Am. J. Cancer **16**:830, 1932.
84. Hutton, A. J., and Young, A.: Surg., Gynec. & Obst. **48**:333, 1929.
85. Davison, C., and Weil, A.: Arch. Neurol. & Psychiat. **19**:415, 1928.
86. Zollinger, R.: Am. J. Surg. **19**:137, 1933.

portion of the spinal column. Fletcher [87] and later Fletcher, Woltman and Adson [88] described 10 cases of chordoma in the sacrum, in which neurologic manifestations were produced, but reported none of chordoma in the lumbar region.

Chordoma usually is a cellular, slowly growing neoplasm, and frequently it is present a long time before it produces symptoms, but because of its tendency to invade and destroy bone, its complete removal is difficult. This difficulty is accentuated in most instances by the location, which makes the tumor inaccessible, although in a few instances it is encapsulated and can be removed completely. Chordoma has a characteristic gelatinous or mucinous appearance and frequently contains spicules of bone, around which it has grown. Microscopically it has specific characteristics by which it is recognized and which have been summarized by Fletcher, Woltman and Adson as follows: (1) the formation of intracellular and extracellular mucus; (2) the presence of physaliferous or huge vacuolated cells containing mucus; (3) the lobular arrangement of the tumor cells, which usually grow in cords; (4) the occasional occurrence of vacuolation of the nuclei, and (5) the close resemblance to notochordal tissue as seen in the nuclei pulposi of the intervertebral disks.

These same authors pointed out that the production of mucus, which is the most primitive function of notochordal tissue, is also the most important characteristic of notochordal tumors. In the more malignant forms of chordoma the ability to produce intracellular mucus remains to the last. They also pointed out that the criteria on which a diagnosis of malignancy is based are the same for these as for other neoplasms. The mitotic figures and other evidence of activity, such as cellular pleomorphism, hyperchromatic and irregular nuclei and multinucleated tumor giant cells, have been observed by numerous authors as well as themselves. Another point which is at times of some help in arriving at a diagnosis is the presence of glycogen in the cytoplasmic vacuoles of these cells as pointed out by several authors, especially Stewart and Morin [89] and Cappell.[90] However, Adson, Woltman and I [91] found a positive reaction with Best's carmine stain for glycogen, even after the tissue had been fixed in solution of formaldehyde U. S. P., which is a watery solution and in which glycogen is readily soluble. We found that after fixation in absolute alcohol there was more carmine-staining

87. Fletcher, E. M.: Sacrococcygeal Chordomas, Thesis, University of Minnesota Graduate School, 1933.

88. Fletcher, E. M.; Woltman, H. W., and Adson, A. W.: Arch. Neurol. & Psychiat. **33**:283, 1935.

89. Stewart, M. J., and Morin, J. E.: J. Path. & Bact. **29**:41, 1926.

90. Cappell, D. F.: J. Path. & Bact. **31**:797, 1928.

91. Adson, A. W.; Kernohan, J. W., and Woltman, H. W.: Arch. Neurol. & Psychiat. **33**:247, 1935.

material than after fixation in a 10 per cent solution of formaldehyde. It was suggested that at least some of the material was glycogen and some an allied substance, which was designated as paraglycogen.

Sarcoma.—Sarcoma ranks fourth in frequency among tumors of the spinal cord. Rasmussen, Adson and I found that 55 tumors (10 per cent) of our entire series of 557 were sarcoma. Represented in this group were lymphosarcoma, myelosarcoma, giant cell sarcoma, Hodgkin's disease and osteogenic sarcoma. Eleven per cent were cervical, 56 per cent thoracic, 22 per cent lumbar and 11 per cent sacral in location. Most of these neoplasms arose from bone, cartilage, extradural fat and connective tissue; 91 per cent were extradural, 5 per cent were both extradural and intradural and 4 per cent were intradural but extramedullary. This is such a mixed collection of tumors, and each group is so small and the type so rare, that further review seems to be superfluous in view of the fact that all the types represented are described in general textbooks and studies on tumors in general.[92] There is perhaps one type which calls for special mention, namely, primary lymphoblastoma of the spinal dura. Some of the tumors of this type may be local manifestations of a disease process from other parts of the body, but unquestionably some are primary, and tissue from which they arise is not clear. Normally, lymphoid tissue is not present in or around the spinal dura, although lymphosarcoma and Hodgkin's lymphoblastoma have been found there in the form of comparatively small localized masses as a primary growth which responds to surgical removal followed by roentgen therapy, or to biopsy for identification and subsequent roentgen irradiation, in a characteristic and spectacular manner. Most of the other types of sarcoma found in the series can be explained readily as originating from preexisting tissue, for example, myeloma and osteogenic sarcoma from the vertebrae.

Metastatic Tumors.—Tumor metastases in the spinal cord are not common, and I have been unable to find any adequate statistical studies relative to their frequency. My own experience with intraspinal metastatic neoplasms, however, has been limited; there is one phenomenon which I have observed in connection with such metastases, namely, extensive myelomalacia. Occasionally, around a metastatic nodule in the brain a variable zone of softening is present; this zone usually is narrow. In the spinal cord the region of myelomalacia is often extensive; it sometimes extends over many segments of the spinal cord. There is no adequate explanation for this phenomenon. The most common tumor to metastasize to the spinal cord is carcinoma of the lungs, which seems to have a predilection for the central nervous system. Metastasis to the spinal dura from various sources, such as the lungs,

92. Ewing, J.: Neoplastic Diseases: A Treatise on Tumors, ed. 3, Philadelphia, W. B. Saunders Company, 1928.

breasts, prostate gland and so forth, is much more common than metastasis to the spinal cord itself. A metastasis in the dura causes compression of the spinal cord but, as a rule, the cord itself is not invaded. Occasionally, extensive associated myelomalacia occurs. It is strange that neurofibroma and meningioma cause compression of the spinal cord with distortion out of all proportion to the associated dysfunction. On removal of these neoplasms, function usually returns rapidly. A comparatively small metastatic carcinoma usually produces local destruction or myelomalacia of the spinal cord out of all proportion to its size. In many cases carcinoma metastasizes to the bodies of the vertebrae, causing collapse of the bone with distortion and sometimes compression of the spinal cord itself. Carcinomatous meningitis is an unusual complication and is difficult to differentiate clinically from other forms of meningitis.

Granulomatous Lesions, Echinococcus Cyst, Abscesses, Paget's Disease and Other Lesions.—Granulomatous lesions of the spinal cord and its meninges are not common. Rasmussen, Adson and I found 7 tuberculous growths in our series of 377 nonneoplastic intraspinal lesions. However, this large group included a series of so-called protruded intervertebral disks, which I mentioned in a previous paragraph and which I have not considered in this discussion.

In addition to these tuberculous growths there were 1 echinococcus cyst, 4 extradural abscesses and 1 intraspinal abscess. Paget's disease, with its destruction of a body of one or the bodies of several vertebrae and the subsequent collapse of these vertebrae, may produce pressure on nerve roots or even compression of the spinal cord itself. Craig, Dockerty and Harrington [93] recently reported the removal of an intervertebral and intrathoracic blastomycoma simulating a dumbbell tumor. The chief danger of dealing with tuberculous and other granulomatous conditions of the spinal cord and its meninges is the tendency for the organisms to invade the subarachnoid space and produce specific meningitis, especially if only a portion is removed or if the growth is removed piecemeal. Perhaps the advent of some of the newer chemotherapeutic agents may reduce this hazard and give the surgeon a better opportunity to relieve compression of the spinal cord.

Occasionally, inflammation in the subarachnoid space brings about fibrosis and adhesions in this space and impedes or even blocks completely the circulation of cerebrospinal fluid. This condition frequently is referred to as adhesive or cystic arachnoiditis, and from the clinical or surgical points of view it may simulate a neoplasm closely, so much so that operation may be undertaken for its relief. The fibrosis and adhesions in the subarachnoid space are usually of nonspecific inflam-

93. Craig, W. McK.; Dockerty, M. B., and Harrington, S. W.: South. Surgeon 9:759, 1940.

matory origin. Microscopic study of the tissues usually reveals little or no residual inflammation. When the inflammatory process is more or less restricted to the spinal cord, as myelitis, or to the nerve roots, as radiculitis, the disease process can be distinguished from neoplastic processes by studies of the cerebrospinal fluid, by pressure of the jugular veins and even by roentgenographic studies with the aid of a radiopaque oil; the latter procedure usually is not resorted to, because of the irritating effects of the iodized oil on the previously inflamed meninges. These inflammatory processes usually are nonspecific, and histologic studies of the tissues are not illuminating as to the duration or the type of inflammatory process.

TECHNIC

It is unnecessary to discuss in much detail the staining methods employed in neurosurgical pathology. They have been described in many textbooks on histologic technic and in some textbooks of neuropathology. In addition, there are several excellent small books on neurohistologic technical procedures. However, several points should be emphasized.

There seems to be some difference of opinion as to the value of diagnosis of fresh tissue, especially in relation to tumors of the brain and spinal cord, and also as to the merits of several methods available. I have been accustomed to the use of polychrome methylene blue and frozen sections of fresh tissue. I have found this eminently satisfactory, and there is no doubt that accuracy in the interpretation of sections prepared in this manner increases with experience. It is possible to make a correct diagnosis in 97 or 98 per cent of the cases in which a tumor is examined; after a little experience, assistants in the laboratory are correct in their diagnosis in more than 90 per cent of the cases in which a neoplasm is examined. We at the Mayo Clinic prefer fresh frozen sections and polychrome methylene blue, because the structure of the tissue and the relationships of cells to one another and to the blood vessels are preserved. The method of making tissue smears and staining with neutral red as employed by Eisenhardt [94] has the advantage of giving a view of the entire cell rather than of a portion of it. The method of Russell [95] has the advantage of giving more permanent results. An accurate histologic diagnosis of a tumor of the spinal cord can readily be arrived at in practically every case with sections of fixed tissue routinely stained with hematoxylin and eosin. Small portions of the tumor should be placed routinely in Zenker's and Cajal's fixing solutions for special stains in order to confirm the diagnosis. These fixing solutions and the routine 10 per cent solution of neutral formaldehyde are adequate for almost all staining methods needed or called for.

94. Eisenhardt, L., and Cushing, H.: Am. J. Path. **6**:541, 1930.
95. Russell, D. S., and Cairns, H.: J. Path. & Bact. **33**:383, 1930.

COMMENT

There has been much interest in recent years in heredity in relation to tumors in general and carcinoma in particular, but there has been little evidence that heredity is related to tumors of the central nervous system and even less that it is related to tumors of the spinal cord. This is chiefly because of the comparative infrequency of these tumors and the difficulty of obtaining reliable data. It is only during the last generation that surgical removal of tumors of the spinal cord has been undertaken with any degree of regularity, and thus little evidence has been obtained in regard to the presence and type of tumor. Some tumors may be congenital and not give evidence of their presence for many years; however, this has not been proved. Tumors of the spinal cord are rare among infants and children; however, teratoma is found almost exclusively among children, and, as this tumor is an evidence of malformation, it can be assumed to be congenital. Most evidence at present supports the idea that syringomyelia is due to malclosure of the posterior septum of the spinal cord. Many, if not all, blood vessel tumors are the result of a malformation which progresses slowly and undergoes neoplasia; on this basis they can be considered congenital. The tumors and malformations of Lindau-von Hippel disease generally are accepted as not only congenital but familial. According to Penfield and Young, von Recklinghausen's disease is familial, seeming to reappear in succeeding generations according to the mendelian law, and is assumed to be the result of a defect in the germ plasm. There is no evidence for a familial or congenital origin of glioma, meningioma or the solitary type of neurofibroma.

It is extremely difficult to obtain information as to the frequency of tumors of the spinal cord, either in absolute numbers or in numbers relative to tumors of the brain. Cushing and Eisenhardt reported on 330 tumors diagnosed as meningioma; of these, 18 (5.4 per cent) were found in the meninges of the spinal cord. At the Mayo Clinic, Brown studied 130 tumors of the spinal cord, and Turner, Craig and I studied 370 tumors of the brain, all diagnosed as meningioma. Of these 500 tumors of the central nervous system grouped as meningioma, 130 (26 per cent) were of the spinal cord. In our entire collection of 3,256 tumors of the central nervous system, at present 621 (19 per cent) are tumors of the spinal cord. This high percentage of tumors of the spinal cord is fortuitous. I doubt if it is a true representation of their frequency among the population as a whole, and I doubt if other pathologic laboratories or neurosurgical clinics would consider these figures representative of the relative frequency of tumors of the central nervous system.

Notes and News

Finney-Howell Research Foundation, Inc.—Announcement has been made that all applications for fellowships for next year must be filed in the office of the foundation, 1211 Cathedral Street, Baltimore, by Jan. 1, 1942. Applications received after that date cannot be considered for 1942 awards. The awards will be made March 1, 1942. Fellowships carrying an annual stipend of $2,000 are awarded for the period of one year, with the possibility of renewal up to three years. When deemed wise by the board of directors, special grants of limited sums may be made to support the work carried on under a fellowship. Applications must be made on the blank form which will be furnished by the secretary or any member of the board of directors.

Awards.—The Sedgwick Memorial Medal of the American Public Health Association has been awarded to Charles Armstrong of the United States Public Health Service for his research in the field of virus diseases.

Nominations are solicited for the 1942 award of $1,000 established by Mead Johnson & Company to promote researches dealing with the B complex vitamins. The recipient of this award will be chosen by a committee of judges of the American Institute of Nutrition. Communications should be addressed to Arthur H. Smith, secretary, Wayne University College of Medicine, Detroit. The formal presentation will be made at the annual meeting of the institute at Boston on April 1.

University News.—W. A. D. Anderson, formerly instructor in pathology at the University of Tennessee, is now assistant professor of pathology in St. Louis University.

Medical Activities Coordinated.—A new division of medical sciences has been established at the University of North Carolina, Chapel Hill, to include the school of medicine, the school of public health, the university health service and the tricounty (Orange, Person and Chatham counties) health service. W. R. Berryhill, dean of the medical school, is chairman of the new division.

Fellowships in Nutrition.—Swift & Company, Chicago, have established at universities and medical schools a limited number of fellowships for research in nutrition. To be eligible for grants, projects should be aimed at one of the following objectives: (1) the development of fundamental information on the nutritive properties of foods; (2) the application of this fundamental information on the nutritive properties of foods to the improvement of the American diet and health. For further information address Dr. R. C. Newton, Research Laboratories of Swift & Company, Union Stock Yards, Chicago.

Book Reviews

Lymphatics, Lymph, and Lymph Tissue. Their Physiological and Clinical Significance. Cecil Kent Drinker and Joseph Mendel Yoffey. Harvard University Monograph in Medicine and Public Health, Number 2. Pp. 406. Price, $3.50. Cambridge, Mass.: Harvard University Press, 1941.

This is a timely book; there has long been need for a unit embracing compilation, critical analysis and correlation of the large mass of physiologic, anatomic and clinical data relating to lymphatics, lymphoid organs and lymphocytes. This book meets reasonable requirements within the limits of definite objective evidence. The presentation of data relative to blood and lymph capillaries, with special reference to contractility, dilatation and permeability to colloids and crystalloids under normal and experimental conditions, as regards especially the relation to lymphedema and hypertension, seems complete and logical. The fundamental mechanism of the blood capillary–tissue space–lymphatic capillary association receives repeated emphasis in explanations of the primary function of the lymphatics as channels for the removal from the tissues of materials which are not absorbed by the blood vessels. Clinicians will find this portion of the book especially interesting and helpful.

The sections on the lymphoid organs and on the significance of the lymphocytes are relatively less satisfactory. This follows from the more speculative interpretation of much of the recorded observations. As regards the lymphocytes, lack of conviction is acknowledged in the "Preface": ". . . the essential functions of these apparently indispensable cells still escape us." Nevertheless, the various suggestions regarding function, with the supporting evidence, are fairly presented while more or less sketchily appraised. It is alleged that the lymphocytes function in fat metabolism, in protein metabolism, in carbohydrate metabolism, as blood cell ancestors, as vehicles for the transport of viruses, in immunity reactions and in the neutralization of intestinal toxins. The authors weigh the evidence for a migration of lymphocytes through the intestinal mucosa as an explanation for the daily enormous loss of lymphocytes from the body, and express doubt of its validity.

One gets the impression that they incline to the belief that one of the important functions of the lymphocytes is to serve as carriers of viruses, especially the vaccinia virus. They seem to incline also toward the monophyletic interpretation of blood cell origin and to the recognition of the small lymphocyte as an embryonal cell, relatively undifferentiated, with multiple developmental potentialities, one of which is to produce erythrocytes and granulocytes. While definitely accepting the fact, for almost the first time among pathologists and physiologists, that the normal bone marrow contains an abundance of lymphocytes, occasionally even in the form of nodules, they leave open the question of origin; they apparently favor the idea that the cells are filtered out of the blood stream. In view of this position is regrettable that comparative data receive such brief consideration, especially as relates to the bone marrow of birds. They assess the significance of the alleged discriminations among "primitive cells" of certain investigators, "lymphoid cells" and small lymphocytes in bone marrow, as based "mainly on subjective grounds." In the marrow of birds the evidence that lymphocytes are identical with "primitive cells" seems conclusive.

Birds lack lymph nodes; this lack is compensated for by the occurrence of many lymph nodules scattered throughout the marrow. There is here an organ in which lymphoid and myeloid tissues are intimately associated. Study of this marrow reveals that the small lymphocytes enter the abundant capillaries of the nodules whence they are carried to the extranodular venous sinuses where they grow in size, acquire the cytologic features of hemocytoblasts and produce

erythrocytes. Small lymphocytes also migrate into the extranodular stroma where, after a period of growth into hemocytoblasts, they mature into granulocytes. A citation of the evidence with reference to the marrow of birds would certainly have given much more adequate support to the authors' cautious inclination regarding the significance of the lymphocyte as a blood cell ancestor. The text includes 45 tables, in some of which are assembled observations covering a wide field, e. g., table 20 with the heading "Comparative Chemical Composition of Blood Serum and of Lymph in Various Animals." There are also 50 instructive charts and figures and a 44 page bibliography. The extensive tables are a valuable feature of the book.

Recognizing that the authors did all that was possible with the confused literature on the significance of the lymphocyte, one feels moved to express amazement that so little attention has been paid by pathologists to so vital a problem. In view of the widespread occurrence of extramedullary blood formation, involving the lymphocytes in lymph nodes, the spleen and the liver in certain anemias, leukemias and neoplastic metastases, and the prevalence of "round cell infiltration" it seems that a concentrated correlated attack on the problem of the function of the lymphocyte would long since have been organized by pathologists.

The authors emphasize a point heretofore largely ignored in discussions of the functional significance of the lymph-vascular system, namely, the essential independence of lymphoid tissue from the lymph stream. Only in mammals is lymphoid tissue found in the form of lymph nodes topographically associated with the lymph vascular system, and even here the relationship is essentially fortuitous. Since lymphocytes ultimately are delivered to the blood vascular system, their association as aggregations would be equally effective whether adjacent to venous sinuses (as in the spleen) or to lymph sinuses. In discussing the function of the lymph nodes the authors favor the view that the nodes serve as mechanical filters for bacteria, senile red corpuscles and tissue debris. At the same time they indicate that in certain clinical cases "as far as barrier action is concerned, the patient would perhaps be better off had he possessed neither lymphatic vessels nor nodes."

The Avitaminoses. The Chemical, Clinical and Pathological Aspects of the Vitamin Deficiency Diseases. Walter H. Eddy, Ph.D., professor of physiological chemistry, Teachers College, Columbia University, New York, and Gilbert Dalldorf, M.D., pathologist to the Grasslands and Northern Westchester Hospitals, Westchester County, New York. Second edition. Cloth. Pp. 519, with 69 illustrations. Price $4.50. Baltimore: The Williams & Wilkins Company, 1941.

The second edition of the textbook on the involved subject of the avitaminoses has won its spurs as a standard reference. The authors indicate the necessity for a second edition by "a complete rewriting of most of the text." The functions of the text from all viewpoints, clinical, chemical and pathologic, have been preserved to the specialist as well as to the practitioner. The authors' modesty in the restatement of the preface to the first edition that "The book has been planned to be a helpful manual rather than a complete treatise" is a commendable understatement of fact. In general, the historical development does not measure up to the standard of the text as a whole.

The organization of the subject matter by and large is quite logical. At times the reader finds the inclusion of certain clinical materials anticipating the pathologic discussion rather disconcerting, but this is a minor fault. The authors show little tendency to support the movement for the utilization of gravimetric methods to the exclusion of biologic units, so that the clinician is still confronted with the necessity of maintaining a working knowledge of both.

Thiamine is consistently referred to as the antineuritic vitamin, and the work of Phillips controverting this position is given short shrift (p. 172). It is interesting to note the growing clinical evidence supporting the contention that thamine is not the sole antineuritic factor. In Brown's recent observation (Boston) a control

group of patients with alcoholic polyneuritis had no longer hospital stay than those receiving full doses of thiamine hydrochloride. The evidence of a vitamin B_1 responsibility for degenerative changes in the nerve cells of the plexuses and resultant pyloric thickening and hypertrophy is not well established (p. 199).

Occasional minor errors creep into the text. On page 249 the reference is made to Rhoads' rather than to Miller and Rhoads' studies as clearly given in the bibliography. On page 253 porphyrinemia is cited in pellagra although later attention (p. 290) is directed to Watson's refutation of this point. Casal is spelled "Casel" on the plate opposite page 278 and page 279. The recent work of Lund and Crandon gives the incubation time for scurvy as five months, although the ascorbic acid level of the plasma was zero in forty-two days and that of the white blood cells zero in one hundred and twenty-two days. These figures are somewhat at variance with the earlier authorities cited in the text. The effort to attribute all hemorrhages of typhoid fever to vitamin C deficiency is rather gratuitous in that epistaxis at least is a prodromal symptom of the disease. The chapter entitled "Vitamins and Infectious Diseases" is a very judicial one. Due weight is given to the available evidence, but conditioning factors are not overlooked. The concludng chapters on the laboratory tests and the vitamin values of foods are particularly helpful.

In the judgment of the reviewer this volume is one of the most valuable of recent texts. It should be on the "must" list of all thoughtful physicians.

Photodynamic Action and Diseases Caused by Light. Harold Francis Blum, Ph.D., The Washington Biophysical Institute (formerly associate professor of physiology, University of California Medical School). American Chemical Society Monograph Series. Cloth. Pp. 309, with 50 illustrations. Price $6. New York: Reinold Publishing Corporation, 1941.

The vast extent of research in the supposedly restricted field of photodynamic action and the even greater volume of literature which has accumulated concerning diseases caused by light are not generally realized. Instead of merely reviewing all this, the author in this book correlates and systematizes the various phases of the work. Because of his wide experience in this field, he is exceptionally well qualified to write on subjects pertaining to it. He divides the volume into four parts. In the introduction he discusses in detail some of the fundamental principles of the nature of radiation as well as its general biologic effects, and this introduction is not only indispensable for those unfamiliar with the subject, but is an excellent review for those who already have definite interests along these lines. In the second part the author is concerned with various aspects of photodynamic action, and such subjects as the mechanism, the role of oxygen and the factors determining photodynamic action are given thorough consideration. In the third part he discusses the diseases of domestic animals produced by light. Such maladies as hypericism, geeldikkop and the more familiar buckwheat poisoning are included, and the discussion of these should be of especial interest to veterinarians as well as others concerned with animal husbandry. In the fourth part, which is a considerable portion of the volume, he is concerned with the various diseases in man which are caused by light. He includes a full discussion of sunburn and the photosensitization of skin to various drugs, cosmetics and porphyrins, as well as a consideration of precancerous and cancerous lesions due to sunlight. Various other skin diseases produced by or aggravated by light are also included. The book contains numerous tables and figures, is carefully documented and is also readable. While this book should be read by all who are concerned with photodynamics and photosensitization, members of the medical profession interested in diseases of the skin will also discover in it much valuable information.

Books Received

DISEASES OF THE BLOOD AND ATLAS OF HEMATOLOGY, WITH CLINICAL AND HEMATOLOGIC DESCRIPTIONS OF THE BLOOD DISEASES INCLUDING A SECTION ON TECHNIC AND TERMINOLOGY. Roy R. Kracke, M.D., professor of bacteriology, pathology and laboratory diagnosis, Emory University School of Medicine; pathologist to the Emory University Hospital; consultant in hematology to the Grady Hospital and Eggleston Hospital for Children, Atlanta, Ga.; formerly director of the hematological registry, American Society of Clinical Pathologists. Second edition, thoroughly revised, reset and enlarged. Pp. 692, with 54 color plates and 46 other illustrations. Price $15. Philadelphia: J. B. Lippincott Company, 1941.

ABOUT OURSELVES: A SURVEY OF HUMAN NATURE FROM THE ZOOLOGICAL VIEWPOINT. James G. Needham, with illustrations by William D. Sargent. Pp. 276. Price $3. Lancaster, Pa.: The Jaques Cattell Press, 1941.

THE MICROBE'S CHALLENGE. Frederick Eberson, Ph.D., M.D., director of laboratories, pathologist, Gallinger Hospital, Washington, D. C. Pp. 354. Price $3.50. Lancaster, Pa.: The Jaques Cattell Press, 1941.

ANNUAL REPORT FOR THE YEAR 1940 OF THE SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH, JOHANNESBURG. E. H. CLUVER, M.A., M.D., B.Ch., D.P.H., F.R.S.I., director. Pp. 67. Johannesburg: The South African Institute for Medical Research, 1941.

FUNCTIONAL PATHOLOGY. Leopold Lichtwitz, M.D., chief of the medical division of the Montefiore Hospital; clinical professor of medicine, Columbia University, New York. Pp. 567, with 198 illustrations. Price $8.75. New York: Grune & Stratton, 1941.